Substrate utilization by intestinal mucosal tissue strips from patients with inflammatory bowel disease

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Jørgensen, Jimmy, and Per Brøbech Mortensen. Substrate utilization by intestinal mucosal tissue strips from patients with inflammatory bowel disease. Am J Physiol Gastrointest Liver Physiol 281: G405–G411, 2001.—A primary metabolic disorder may be present in the colonic mucosa of patients with ulcerative colitis. Preserving the epithelium in situ, we evaluated the metabolism of the colonic mucosa of control patients and patients with ulcerative colitis and Crohn’s disease. Colonic mucosal strips (~500 mg) were incubated with partially 14C-labeled acetate (C2), butyrate (C4), hexanoate (C6), octanoate (C8), and glucose, and the production of CO2 and ketone bodies was quantitated. Metabolism by small intestinal mucosal strips was also evaluated. Compared with controls, no decrease in either CO2 or ketone body production by colonic strips from patients with either ulcerative colitis or Crohn’s disease was observed for any substrate. The CO2 production from each of the C2–C8 fatty acids was the same for colonic and small intestinal strips, whereas CO2 production from glucose was higher in small intestinal strips than in colonic strips. The production of ketone bodies was low in small intestinal strips. A primary metabolic disorder in the colonic mucosa of patients with inflammatory bowel disease was not found.

ulcerative colitis; Crohn’s disease; butyrate; oxidation

SHORT-CHAIN FATTY ACIDS (SCFAs) are produced by bacterial fermentation of unabsorbed dietary fibers and proteins reaching the colonic lumen. SCFAs are the main fuels of the colonic mucosa, and they play an important role in maintaining its structure and function (16). Accordingly, decreased mucosal metabolism of SCFAs has been linked to various disease states of the colon, such as ulcerative colitis (30) and diversion colitis (20), and to the cytokinetic changes taking place during the tumorigenic process of colon cancer (41).

Most studies of colonic mucosal metabolism have been done with isolated epithelial cells (colonocytes) (17, 23, 29, 30). However, as recently shown by Fitch and Fleming (15), data obtained from such in vitro studies do not appear to accurately reflect SCFA mucosal metabolism in vivo. This group used a newly developed colonic perfusion model of the anesthetized rat, and their results indicate that the apparent Michaelis-Menten constant (Km) for butyrate oxidation to CO2 as well as the concentration needed to achieve maximal oxidation (Vmax) are much higher for the colonic mucosa in vivo than for isolated colonocytes (8–10, 23, 25).

In vivo studies of colonic mucosal metabolism are difficult to perform in humans. To approximate the physiological environment, routine coloscopic biopsies (~15 mg) have therefore been used (6, 14). As for in vivo studies, this method allows for metabolic studies of the colonic epithelium in situ and could represent a favorable means of estimating mucosal metabolism in the human colon in vivo. However, the coefficient of variance for the biopsy method is high (6), and determination of Km and Vmax for the oxidation of SCFAs does not appear to be possible (14).

The idea that ulcerative colitis represents an energy deficiency disease of the colonic epithelium has proven controversial as judged by studies using isolated colonocytes or biopsies (5, 9, 12, 14, 30). Because data obtained with these methods have been challenged, we used our novel method (24) of large (~500 mg) human colonic mucosal strips to further investigate the metabolic activity in presumably healthy mucosa as well as in mucosa from patients with ulcerative colitis and Crohn’s disease. This method is highly reproducible, with a coefficient of variance <10%. In addition, kinetic data of substrate oxidation by mucosal strips appear to resemble those obtained in vivo (15, 24), indicating that information on actual in vivo metabolism may be achieved with the use of mucosal strips. Studies of small intestinal strips were included for reasons of comparison.

MATERIALS AND METHODS

Materials

Bovine serum albumin (fraction V), nonlabeled hexanoate, d-glucose, and [1-14C]acetate and [1-14C]hexanoate were obtained from Sigma Chemical (St. Louis, MO). Nonlabeled acetate and butyrate were obtained from BDH (Poole, UK). [1-14C]butyrate and [1-14C]octanoate were obtained from Du Pont-NEN Research Products (Boston, MA). Nonlabeled octanoate was obtained from Merck Chemical (Darmstadt, Ger-

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many). [6-14C]glucose was obtained from Amersham (Little Chalfont, UK).

**Patients**

**Control patients.** Two men and four women (age 30–79 yr, mean 63 yr) were included. Four patients had adenocarcinoma of the cecum, colon, or rectum; one had diverticulosis of the sigmoid colon, and one suffered from severe constipation. Sampling sites were cecum (n = 1), ascending (n = 2) and sigmoid (n = 2) colon, and rectum (n = 1).

**Patients with ulcerative colitis.** Five men and two women (age 21–50 yr, mean 35 yr) were included. The disease activity was mild (n = 4) or moderate (n = 3) (37). Medical treatment was oral: corticosteroids only (n = 4), mesalazine only (n = 2), or both (n = 1). The main operative reasons were dependence on corticosteroids (n = 3), increased risk of malignancy in longstanding disease (n = 2), and ineffectiveness of medical treatment (n = 2). Sampling sites were cecum (n = 1) and ascending (n = 2), transverse (n = 2), and sigmoid (n = 2) colon.

**Patients with Crohn’s disease.** Four men and three women (age 15–72 yr, mean 34 yr) were included. Patients had mild to moderate diarrhea (up to 5 movements a day) and were orally treated with corticosteroids (n = 3), azathioprine (n = 1), or mesalazine (n = 1) or were not treated (n = 2). The main operative reasons were dependence on corticosteroids (n = 2), ineffectiveness of medical treatment (n = 3), or fistulae (n = 2). Sampling sites were cecum (n = 4) and ascending (n = 2) and sigmoid (n = 1) colon.

**Patients with small intestinal disease.** Two men and three women (age 16–65 yr, mean 41 yr) were included. Three of these patients suffered from Crohn’s disease and had no diarrhea (n = 2) or jejunostomy (n = 1). Medical treatment was oral corticosteroids (n = 1), azathioprine (n = 1), or none (n = 1), and main operative reasons were ileal or jejunal stenosis. In addition, one patient suffered from an ileocecal carcinoid tumor and one suffered from intestinal lymphoma. Sampling sites were terminal (n = 3) and proximal (n = 1) ileum and distal jejunum (n = 1).

**Isolation and Incubation of Mucosal Strips**

The warm ischemia time of resected colonic segments was <5 min. Immediately after resection, the segments were cut open and washed clear of debris with water. Within the same area, mucosal strips (wet wt 505 ± 9 mg; mean ± SE) were then removed from the muscularis propria by means of sharp scissors and forceps. Strips were placed in Krebs-Henseleit saline (26) previously gassed with O2-CO2 (19:1 vol/vol) and immediately transported to the laboratory. One mucosal strip was incubated in 25-ml conical flasks with Krebs-Henseleit saline containing 2.5% (wt/vol) bovine serum albumin and the appropriate substrates (total volume 2 ml), and incubation for 40 min was done as previously described for isolated colonocytes (34), with the exception that hyaluronidase was omitted. In each experiment, identical control incubations from which exogenous substrate was omitted as correction for the nonspecific radioactivity and baseline ketogenesis were run in parallel. Metabolism was determined in three adjacent strips for each substrate at a concentration of 5 mmol/l. All experiments were done consecutively, i.e., the order of experiments (colonic and small intestinal) was determined by the disease (cancer, Crohn’s disease, ulcerative colitis, etc.) of the patients who were admitted to our operating theater.

**Histological Assessment of Resected Intestinal Segments**

**Colon segments from control patients.** In control patients, strips were taken at least 5 cm from macroscopically affected mucosa. For the four patients with adenocarcinoma, microscopy did not reveal signs of malignancy or dysplasia in areas of mucosal sampling or resection borders. For the patient with diverticulosis, a mild degree of mucosal melanosis was found in some areas, and for the patient suffering from constipation, submucosal fibrosis was found throughout the colon. Inflammatory changes in the areas of sampling were not reported for any of the control patients.

**Colon segments from patients with inflammatory bowel disease.** Because the aim of the present study was to investigate the possible presence of a primary metabolic disorder in the mucosa of patients with inflammatory disease, mucosal sampling was only done in the areas least affected. Therefore, the epithelial layer of strips was always macroscopically intact, and strips were only included if they had been removed easily from the submucosal layer.

In all cases, an overall histological assessment of the activity of resected segments was done. Active inflammation was defined as the presence of ulcerations and/or crypt abscesses.

For patients with ulcerative colitis, varying degrees of chronic inflammation were present in resected segments from all patients. In segments from five of seven patients, signs of active inflammation were present.

For patients with Crohn’s disease, varying degrees of chronic inflammation were present in resected segments from all patients. In segments from six of seven patients, signs of active inflammation were present.

**Small intestinal segments.** For two of the three patients with Crohn’s disease, varying degrees of chronic inflammation as well as signs of active inflammation were present in resected segments, and for the third patient (operated for jejunal stenosis), histological assessment of the resected segment was not done. For the patient suffering from an ileocecal carcinoid tumor, borders of the resected segment were normal. For the patient suffering from intestinal lymphoma subepithelial infiltrates of malignant (T cell type) lymphocytes were present in most areas having intact epithelium, and in some areas epithelial ulcerations were seen.

**Validation Experiments**

**Intersample coefficient of variance (reproducibility).** Intersample coefficient of variance previously evaluated for colonic mucosal strips was 9.6% for the production of CO2 from butyrate oxidation (24). Similarly, the coefficient of variance for the production of CO2 evaluated for nine strips taken from immediately adjacent areas of ileal mucosa from a 17-year-old female patient with Crohn’s disease was 9.6%.

**Tissue viability.** The production of CO2 from butyrate oxidation by colonic mucosal strips was shown previously to be linear with time for at least 80 min (24). In the present study, three strips were taken for each time point tested (20, 40, 60, and 80 min) from immediately adjacent areas of ileal mucosa from a 34-year-old woman with ulcerative colitis. The oxidation of butyrate to CO2 by strips was linear for at least 80 min (R = 0.98; P = 4.6 × 10^-3), and the x-axis intercept was not significantly different from the origin. This indicates efficient metabolic performance during the 40-min incubation period used for experiments in the present study.
Radioactivity was counted in a model 4530 Packard Tri-Carb liquid scintillation spectrometer (Packard Instrument), and counts were corrected for nonspecific activity as mentioned in Isolation and Incubation of Mucosal Strips. After protein precipitation of the incubation medium, supernatants were neutralized to pH 7.4 with 20% KOH. The amounts of β-hydroxybutyrate and acetocetate were determined spectrophotometrically (LKB Biochrom 4050 spectrophotometer, Pharmacia Biosystems, Copenhagen, Denmark; Refs. 28 and 39). Absorbance changes were corrected for parallel changes in reagent blanks. The term “ketone bodies” refers to the net production of acetocetate plus β-hydroxybutyrate.

Calculations

Generation of CO₂ from the oxidation of 14C-labeled substrates was calculated from the specific activities of substrate solutions and trapped 14CO₂ in NaOH. The values of CO₂ production for fatty acids and glucose correspond to the oxidation of 1-C and 6-C substrate carbons, respectively, and calculations of CO₂ and ketone body production were based on the mean value of three adjacent strips. The values of ATP production were calculated on the assumption that fatty acids were completely oxidized, i.e., on the basis of acetate, butyrate, hexanoate, and octanoate providing 10, 27, 44, and 61 mol ATP/mol, respectively (36). Results are expressed as nanomoles per minute per gram of wet weight of mucosal strips.

Statistical Analysis

Because of unequal variance among substrates and the fact that not all data were normally distributed, nonparametric statistics were performed in this study. When the metabolism of different substrates was compared within groups (colorectal and small intestinal), Friedman’s repeated-measures ANOVA on ranks was used. If statistical difference ($P < 0.05$) was found, the Student-Newman-Keuls method was used post hoc for pairwise comparisons. Comparisons between different groups were performed by the Kruskal-Wallis one-way ANOVA on ranks, and Dunn’s method (for unequal group sizes) was used as a post hoc test for pairwise comparisons. The Mann-Whitney rank-sum test was used to compare the metabolism of substrates in relation to age and sex and to compare the metabolism by colonic and small intestinal strips. Values are expressed as medians (25th–75th percentiles).

Ethics

This study was approved by the Ethics Committee for Medical Research in Copenhagen and conducted in accordance with the Declaration of Helsinki.

RESULTS

Oxidation of C2–C8 Fatty Acids and Glucose by Colorectal Strips From Patients With Ulcerative Colitis and Crohn’s Disease

The CO₂ production from each of the substrates: acetate (C₂), butyrate (C₄), hexanoate (C₆), octanoate (C₈), and glucose by colorectal strips from patients with ulcerative colitis and Crohn’s disease did not differ significantly from the production of CO₂ by colorectal strips from control patients (Table 1).

### Table 1. Rate of CO₂ production by groups of colorectal mucosal strips from controls and patients with Crohn’s disease or ulcerative colitis incubated with different substrates

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Controls (n = 6)</th>
<th>CD (n = 7)</th>
<th>UC (n = 7)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetate</td>
<td>23.1 (18.0–26.3)</td>
<td>24.6 (13.5–26.4)</td>
<td>21.7 (15.3–24.6)</td>
<td>0.79</td>
</tr>
<tr>
<td>Butyrate</td>
<td>12.2 (11.3–14.0)</td>
<td>15.8 (11.5–27.9)</td>
<td>21.2 (15.4–24.4)</td>
<td>0.08</td>
</tr>
<tr>
<td>Hexanoate</td>
<td>11.7 (10.3–12.0)</td>
<td>11.2 (10.8–18.7)</td>
<td>12.3 (10.3–17.8)</td>
<td>0.71</td>
</tr>
<tr>
<td>Octanoate</td>
<td>7.2 (6.3–8.0)</td>
<td>7.5 (6.3–12.5)</td>
<td>8.3 (7.7–12.4)</td>
<td>0.23</td>
</tr>
<tr>
<td>Glucose</td>
<td>1.5 (1.0–2.3)</td>
<td>1.0 (0.9–1.4)</td>
<td>1.1 (0.9–1.6)</td>
<td>0.54</td>
</tr>
</tbody>
</table>

Values, in nmol·min⁻¹·g⁻¹, are medians (25th–75th percentiles). The values of CO₂ production for fatty acids and glucose correspond to the oxidation of [1-C] and [6-C] substrate carbons, respectively. Control values for acetate, butyrate, and hexanoate are taken from Ref. 24. All experiments (colonic and small intestinal) presented in this study were done consecutively, see MATERIALS AND METHODS. CD, Crohn’s disease; UC, ulcerative colitis.

Oxidation of C₂–C₈ Fatty Acids and Glucose by Colorectal and Small Intestinal Strips

Because significant differences for the production of CO₂ were not observed among the three clinical groups for any substrate (Table 1), values for each substrate were pooled for statistical analysis (Table 2). The production of CO₂ by colorectal strips was statistically in the order acetate > butyrate > hexanoate > octanoate > glucose. The corresponding order for small intestinal strips was identical, acetate > butyrate ≥ hexanoate ≥ octanoate > glucose. Taking the different energy gain from the complete oxidation of C₂–C₈ fatty acids into account, ATP production for colorectal strips was in the order hexanoate > octanoate > butyrate > acetate, whereas the corresponding order for ATP production by small intestinal strips was octanoate ≥ hexanoate ≥ butyrate ≥ acetate.

The production of CO₂ (and ATP) from the oxidation of each of the substrates butyrate, hexanoate, and octanoate was the same for colorectal and small intestinal strips, whereas acetate oxidation was statistically higher in small intestinal strips. The value of glucose oxidation was approximately five times higher in small intestinal strips (5.7 nmol·min⁻¹·g⁻¹) compared with colorectal strips (1.1 nmol·min⁻¹·g⁻¹).

Production of Ketone Bodies by Colorectal Strips From Patients With Ulcerative Colitis and Crohn’s Disease

Significant differences in the production of ketone bodies by colorectal strips from patients with ulcerative colitis and Crohn’s disease compared with strips from control patients were not found for any substrate (Table 3). Colorectal ketogenesis from acetate and glucose was negative or negligible, whereas ketogenesis from butyrate, hexanoate, and octanoate was ~15–30 nmol·min⁻¹·g⁻¹. Values of ketogenesis were corrected...
Table 2. Rate of CO₂ and ATP production by groups of colorectal and small intestinal mucosal strips incubated with different substrates

<table>
<thead>
<tr>
<th>Substrate (5 mmol/l)</th>
<th>CO₂</th>
<th>ATP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Colorectal (n = 20)</td>
<td>Small intestinal (n = 5)</td>
</tr>
<tr>
<td>Acetate</td>
<td>22.7&lt;sup&gt;a&lt;/sup&gt; (15.4–25.6)</td>
<td>30.2&lt;sup&gt;a&lt;/sup&gt; (27.2–48.0)</td>
</tr>
<tr>
<td>Butyrate</td>
<td>15.4&lt;sup&gt;b&lt;/sup&gt; (11.9–21.7)</td>
<td>17.1&lt;sup&gt;b&lt;/sup&gt; (14.0–28.2)</td>
</tr>
<tr>
<td>Hexanoate</td>
<td>12.0&lt;sup&gt;c&lt;/sup&gt; (10.5–15.3)</td>
<td>12.2&lt;sup&gt;c&lt;/sup&gt; (11.0–14.3)</td>
</tr>
<tr>
<td>Octanoate</td>
<td>7.8&lt;sup&gt;d&lt;/sup&gt; (6.9–10.5)</td>
<td>9.2&lt;sup&gt;d&lt;/sup&gt; (8.5–11.3)</td>
</tr>
<tr>
<td>Glucose</td>
<td>1.1&lt;sup&gt;e&lt;/sup&gt; (1.0–1.7)</td>
<td>5.7&lt;sup&gt;e&lt;/sup&gt; (4.7–8.9)</td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.001</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Values, in nmol·min⁻¹·g⁻¹, are medians (25th–75th percentiles). The colorectal group is based on the pooled data of the 3 colorectal groups (controls, CD, and UC) from Table 1. The values of CO₂ production for fatty acids and glucose correspond to the oxidation of [1-C] and [6-C] substrate carbons, respectively, whereas the values of ATP production were calculated on the assumption that fatty acids were completely oxidized. Values not sharing the same superscript letter within a column are significantly different at P < 0.05. *P values for ATP production are the same as for CO₂ production.

for a positive base value of ketogenesis by strips not supplemented with substrate during incubation. A negative value of ketogenesis therefore indicates substrate inhibition of ketogenesis, possibly combined with an increased consumption of ketone bodies during incubation.

Production of Ketone Bodies from C₂–C₈ Fatty Acids and Glucose by Colorectal and Small Intestinal Strips

As for the CO₂ production (Table 1), significant differences for the production of ketone bodies were not observed between the colorectal groups of strips for any substrate (Table 3), and values for each substrate were subsequently pooled for statistical analysis (Table 4). Ketogenesis from acetate and glucose was negative or negligible both by colorectal and small intestinal strips. Ketogenesis from butyrate, hexanoate, and octanoate by the colorectal strips was significantly higher and in the range of 17–23 nmol·min⁻¹·g⁻¹, which contrasted to the corresponding low production of ketone bodies by small intestinal strips, which were in the range of 2–3 nmol·min⁻¹·g⁻¹. Hence, ketogenesis by small intestinal strips was invariably low, and none of the substrates resulted in a noteworthy increase in the production of ketone bodies by strips from the small intestine.

Colorectal CO₂ and Ketone Body Production in Relation to Regional Site of Sampling, Age, and Sex

The values for the production of CO₂ and ketone bodies from colorectal strips from the three clinical groups were pooled for statistical analysis in Table 5 according to regional site of sampling. Except for glucose, significant differences were not observed in CO₂ production.

Table 3. Rate of ketone body (acetoacetate + β-hydroxybutyrate) production by groups of colorectal mucosal strips from controls and patients with CD or UC incubated with different substrates

<table>
<thead>
<tr>
<th>Substrate (5 mmol/l)</th>
<th>Controls (n = 6)</th>
<th>CD (n = 7)</th>
<th>UC (n = 7)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetate</td>
<td>−3.8 (−3.9 to −2.6)</td>
<td>−1.6 (−4.5 to −1.4)</td>
<td>0.7 (−2.1 to 1.1)</td>
<td>0.11</td>
</tr>
<tr>
<td>Butyrate</td>
<td>23.4 (17.1–37.4)</td>
<td>14.6 (2.8–30.4)</td>
<td>15.9 (5.7–22.7)</td>
<td>0.37</td>
</tr>
<tr>
<td>Hexanoate</td>
<td>31.8 (19.1–41.8)</td>
<td>16.6 (5.8–43.8)</td>
<td>18.8 (9.6–42.2)</td>
<td>0.40</td>
</tr>
<tr>
<td>Octanoate</td>
<td>26.5 (14.1–31.4)</td>
<td>14.0 (−0.2 to 25.9)</td>
<td>16.2 (7.0–31.1)</td>
<td>0.40</td>
</tr>
<tr>
<td>Glucose</td>
<td>−5.1 (−5.9 to −1.9)</td>
<td>−2.5 (−2.9 to −1.5)</td>
<td>−0.9 (−1.9 to −0.9)</td>
<td>0.20</td>
</tr>
</tbody>
</table>

Values, in nmol·min⁻¹·g⁻¹, are medians (25th–75th percentiles). Control values for acetate, butyrate and hexanoate are taken from Ref. 24.
production among regional groups (cecum, ascending and sigmoid colon). As regards the corresponding ketogenesis, significant differences among regional groups were not observed for any substrate. In addition, significant differences were not observed for the production of either CO$_2$ or ketone bodies between the groups of patients aged <40 yr ($n = 10$) and >40 yr ($n = 10$) or between men ($n = 11$) and women ($n = 9$) for any substrate (data not shown).

**DISCUSSION**

A primary metabolic disorder in the colonic mucosa may lead to an energy deficiency state and, subsequently, colonic disease. To evaluate colonic mucosal metabolism in Crohn’s disease and ulcerative colitis, we used large mucosal strips. This method is cumbersome because it requires surgical specimens and does not allow multiple and sequential experiments to be done as the method of coloscopic biopsies does. However, reproducibility is high for the intestinal strip method, which can probably be explained by the uniform sampling of mucosal tissue carefully controlled by using sharp scissors. In contrast, the high variability of form sampling of mucosal tissue carefully controlled by method, which can probably be explained by the uniformity of the method presented in this study is the most reliable. By inflamed mucosa may therefore be secondary to the inflammatory process itself or the use of different techniques (5, 11, 30).

Substrates chosen for this study were 1) acetate and butyrate, abundantly present in the colonic lumen, with the latter considered as the preferred substrate for the colonic epithelium, 2) hexanoate and octanoate, for reasons of fatty acid comparison, and 3) glucose, the universal substrate for mammalian cells. Substrate concentration was 5 mmol/l, which is sufficient to achieve maximum CO$_2$ production ($V_{\text{max}}$) from butyrate oxidation by mucosal strips (24).

### Table 5. Rate of CO$_2$ and ketone body (acetoacetate + β-hydroxybutyrate) production by groups of colonic strips incubated with different substrates according to region of sampling

<table>
<thead>
<tr>
<th>Substrate</th>
<th>CO$_2$</th>
<th>Ketone Bodies</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cecum</td>
<td>Ascending</td>
</tr>
<tr>
<td>Acetate</td>
<td>24.7</td>
<td>23.0</td>
</tr>
<tr>
<td></td>
<td>(12.6–26.3)</td>
<td>(13.0–26.9)</td>
</tr>
<tr>
<td>Butyrate</td>
<td>16.1</td>
<td>19.1</td>
</tr>
<tr>
<td></td>
<td>(12.1–21.8)</td>
<td>(14.0–30.0)</td>
</tr>
<tr>
<td>Hexanoate</td>
<td>11.6</td>
<td>13.5</td>
</tr>
<tr>
<td></td>
<td>(10.7–15.7)</td>
<td>(10.3–19.7)</td>
</tr>
<tr>
<td>Octanoate</td>
<td>7.4</td>
<td>8.7</td>
</tr>
<tr>
<td></td>
<td>(6.0–11.0)</td>
<td>(7.0–13.0)</td>
</tr>
<tr>
<td>Glucose</td>
<td>1.0$^a$</td>
<td>1.7$^b$</td>
</tr>
<tr>
<td></td>
<td>(0.5–1.0)</td>
<td>(1.5–2.3)</td>
</tr>
</tbody>
</table>

Values, in nmol min$^{-1}$$\cdot$g$^{-1}$, are medians (25th–75th percentiles). Ascending, ascending colon; sigmoid, sigmoid colon. Values with different superscript letters within a row are significantly different at $P < 0.05$. The fatty acids used were 1$^4$C labeled, whereas glucose was 6$^{14}$C labeled. This fact may create an inconsistency in the production of CO$_2$ if acetyl CoA molecules entering the TCA cycle are not completely oxidized. However, the main purpose of this study was to compare substrate utilization between controls and patients with Crohn’s disease and ulcerative colitis, which was not affected by the difference in labeling. We could not confirm previous findings of a decreased oxidative capacity for any substrate in the colonic mucosa of either patients with ulcerative colitis or patients with Crohn’s disease (Table 1). Duffy et al. (12) found that in mildly (as opposed to moderately and severely) inflamed mucosa, butyrate and glutamine oxidation was not significantly reduced in ulcerative colitis or Crohn’s disease. Allan et al. (1) found no deficiency of enzyme activity in the β-oxidation pathway of butyrate in the mucosa of patients with ulcerative colitis in histological remission, and Finnie et al. (14) found no defect in colonic mucosal butyrate oxidation in quiescent ulcerative colitis. Furthermore, recent studies estimating in vivo colonic butyrate metabolism indicate that butyrate oxidation is not decreased in quiescent ulcerative colitis (11, 35). In our study, mucosal sampling was only done in least affected areas, sometimes without macroscopic signs of inflammation. Together, these observations suggest that a primary oxidative disorder in the mucosa of patients with inflammatory bowel disease is not present. Previous findings of a decreased ability to oxidize butyrate by inflamed mucosa may therefore be secondary to the inflammatory process itself or the use of different techniques (5, 11, 30).

Colonocytes possess the necessary enzymes for the production of ketone bodies, which may be oxidized by the colonocyte itself (31) or the muscular cells of the intestinal wall (21) or passed on to the liver for oxidation (13). In addition, because they are useful substrates for lipogenesis (phospholipids) in intestinal cells (3), they may play a role in maintaining mucosal

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Integrity. Epithelial denudation of mucosal strips decreases the production of CO\(_2\) (from butyrate oxidation) \(\sim95\%\) (14, 24), and a similar decrement (93%) applies to the corresponding ketogenesis (data not shown), as also shown by Henning and Hird (22). To our knowledge, this study presents the first estimate of ketogenesis by the human colonic epithelium in situ. Whereas ketogenesis from acetate and glucose was negative or absent, ketogenesis from butyrate, hexanoate, and octanoate was substantial and in the same order of magnitude. However, no difference in ketogenesis was found between groups of colorectal strips from control patients and patients with Crohn’s disease and ulcerative colitis for any substrate, which gives additional support to the view that the colonic mucosa in inflammatory bowel disease does not exhibit a defective \(\beta\)-oxidation of C\(_4\)–C\(_8\) fatty acids.

This study also supports previous findings that butyrate does not possess unique oxidative properties (23). From a nutritional point of view (ATP production), the longer-chain SCFA hexanoate and the medium-chain fatty acid octanoate may also serve as excellent substrates for the colonic epithelium (Table 2). Any beneficial effect to the colonic epithelium from these two fatty acids must, however, be provided through rectal instillation, because they are not (or only sparsely) represented in colonic contents. The C\(_2\)–C\(_8\) fatty acids readily enter the mitochondria for oxidation in a carnitine-independent manner, and the ATP production from these substrates was calculated on the assumption that complete oxidation was taking place. For glucose, however, ATP generated via anaerobic glycolysis would not be included in this approach. Thus studies have shown that lactate, provided by anaerobic glycolysis, is a major product of glucose metabolism in isolated rat and human colonocytes (8, 18, 29). Consequently, the ATP production from glucose was not included in Table 2.

CO\(_2\) production from the oxidation of acetate, butyrate, hexanoate, and octanoate by small intestinal strips was roughly the same as for colonic strips (Table 2). This finding is in accordance with previous studies using human ileal biopsies from control patients (12, 14), in which the CO\(_2\) production from butyrate amounted to 50–215% of that of colonic biopsies. In addition, butyrate oxidation by full-thickness ileal tissue specimens from the rabbit has been shown to equal that of colonic specimens (22). Chalfin and Holt (4) found an equal production of CO\(_2\) from octanoate by rat jejunal and cecal full-thickness specimens. In our laboratory, we have done additional studies on the CO\(_2\) production from butyrate oxidation by full-thickness rat intestinal tissue specimens. We found that butyrate oxidation by small intestinal specimens amounted to \(>50\%\) of that of colonic specimens (\(n=6\); unpublished observations). In contrast to these findings, previous studies showed that the ability of rat jejunal or ileal isolated enterocytes to oxidize butyrate is sparse (<10% of that of rat isolated colonocytes; Refs. 17, 25, and 27). An immediate explanation for this discrepancy could be that the terminal ileum of our patients had been subject to colonic metaplasia. However, histological evidence of colonic metaplasia was not found. In addition, metabolic adaptation of biopsy specimens from the terminal ileum after construction of an ileal pouch does not appear to occur for butyrate (7).

Admittedly, caution must be used when comparing data of different studies (different techniques, different denominators in calculations, etc.). Nevertheless, it appears that aspects of fatty acid metabolism are not always predicted from studies using isolated cells but require the use of a model that permits studies of the intestinal epithelium when in situ. Finally, our finding that small intestinal oxidation of glucose was approximately fivefold higher than in colon (Table 2) was not surprising, because glucose oxidation to CO\(_2\) by the small intestinal epithelium is higher than for unesterified fatty acids (40). With the exception of the suckling rat (2, 19), animal studies have shown that ketogenesis in enterocytes is sparse or absent (22, 38). Analogous to those findings, we found that ketogenesis in human small intestinal strips was sparse. A real difference in the regional metabolism of fatty acids by the intestinal epithelium may therefore lie in the ability to produce ketone bodies, which may only be present in the colonic epithelium.

Whereas the production of CO\(_2\) from butyrate oxidation is approximately constant from the cecum to the rectum of the rabbit, the corresponding ketogenesis is more pronounced in the proximal colon (22). Some data suggest that regional variation is not present in the human colon for the CO\(_2\) production from the oxidation of butyrate and glucose (6, 34). As regards ketogenesis, investigation of a possible regional variation in the human colon has previously not been properly addressed, but sparse information indicates that ketogenesis may be higher proximally (32, 33). Data from the present study did not reveal any pattern of regional variation of CO\(_2\) or ketone body production for any substrate in any colorectal group of strips, and the data presented in Table 5 support the view that regional variation of CO\(_2\) and ketone body production does not take place in the human colon.

In summary, this new method using mucosal strips may offer a good estimate of the actual in vivo metabolism by the intestinal mucosa. Using this alternative method, we could not find evidence of a primary metabolic disorder of the colonic mucosa in either Crohn’s disease or ulcerative colitis. From a nutritional point of view, the favorable metabolism of butyrate by the colonic mucosa is not unique. The oxidative and ketogenic properties of the longer-chained fatty acids hexanoate and octanoate are equally good; they are, however, usually not present to any noteworthy extent in colon and must be instilled to have any beneficial effect on the epithelium.

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METABOLISM BY INTESTINAL MUCOSAL STRIPS IN IBD

G411