

Butyrate oxidation is impaired in the colonic mucosa of sufferers of quiescent ulcerative colitis

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Abstract

The short chain fatty acids, acetate, propionate, and butyrate are produced by colonic bacterial fermentation of non-starch polysaccharides. Butyrate is the major fuel source for the colonic epithelium and there is evidence to suggest that its oxidation is impaired in ulcerative colitis. Triplicate biopsy specimens were taken at colonoscopy from five regions of the large bowel in 15 sufferers of ulcerative colitis. These patients all had mild or quiescent colitis as assessed by clinical condition, mucosal endoscopic and histological appearance. The rate of oxidation of glucose, glutamine, and butyrate through to carbon dioxide was compared with that in biopsy specimens from 28 patients who had no mucosal abnormality. Butyrate (272 (199-368)) was the preferred fuel source for the colitic mucosa followed by glutamine (33 (24-62)) then glucose (7.2 (5.3-15)) pmol/ μ g/hour; medians and 95% confidence intervals, $p < 0.01$. There was no regional difference in the rate of utilisation of these metabolites. In the group with colitis the rate of butyrate oxidation to carbon dioxide was significantly impaired compared with that in normal mucosa decreasing from 472 (351-637) pmol/ μ g/hour to 272 (199-368) pmol/ μ g/hour; median and 95% confidence intervals, $p = 0.016$. The rate of glucose and glutamine utilisation were not significantly different between normal and colitic mucosa. These data confirm that in quiescent ulcerative colitis there is an impairment of butyrate oxidation.

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Butyrate is a short chain fatty acid produced by the bacterial fermentation of non-starch polysaccharides (fibre) in the human large bowel.^{1,2} It has been calculated that 500-600 mmol of short chain fatty acids, with a total energy value of 140-180 kcal, are produced each day by a human on an average European diet.³ These short chain fatty acids are rapidly absorbed.⁴ Cummings *et al* studied the difference in molar ratios of short chain fatty acids in the gut lumen and the portal circulation in six sudden death victims.⁵ They found a pronounced drop in the molar ratio of butyrate to the total short chain fatty acid concentration in the portal blood, indicating that it was extensively metabolised in the gut mucosa. Roediger, using isolated colonic epithelial cells (colonocytes) from human resection specimens, showed that butyrate was the major fuel source for these cells, with those from the distal colonic mucosa being more reliant on it than those from proximal mucosa.⁶ He also discovered that colonocytes from sufferers of ulcerative colitis

exhibited an impaired ability to metabolise butyrate.⁷ This led to the hypothesis that ulcerative colitis is characterised by an energy deficiency, its predilection for the distal colon reflecting its greater dependence on butyrate as a fuel source.

Roediger's work can be criticised as there was doubt about the viability of the cell suspensions, 15-30% of the cells exhibited damaged membranes and their oxygen consumption was linear for only one hour. Also, all the patients from whom the colonocytes were isolated had severe colitis needing resection and hence the changes seen in butyrate oxidation may have been secondary to the inflammatory response.

We have developed and validated a radio-tracer technique to study the rate of fuel substrate oxidation in endoscopically obtained biopsy samples. We have shown that in normal large bowel mucosa there is no regional variation in the rate of substrate oxidation.⁸ Using this technique we have investigated the regional variation in the rate of substrate oxidation in colonoscopically obtained mucosal biopsy specimens from 15 sufferers of quiescent colitis and we have compared this with substrate oxidation in biopsy specimens of healthy mucosa from 28 patients. The fuel substrates used were butyrate, glutamine, and glucose. Glutamine was chosen, as it is the preferred fuel substrate for the small intestinal enterocyte and may play a part in the nutrition of the large bowel, especially if there was an inability to utilise butyrate.⁹ Glucose metabolism was measured as it is the ubiquitous fuel source for mammalian cells.

Methods

All materials were of Analar or equivalent grade. Radioisotopes were obtained from Amersham International, Little Chalfont, Buckinghamshire, UK and New England Nuclear, NEN Division, Dreiech, Germany.

PATIENTS STUDIED

Colonoscopic biopsy samples were obtained from 15 patients (10 male, median age 43, range 22-78 years) with quiescent or mild ulcerative colitis. All these patients had suffered from colitis for longer than six months and the diagnosis had been confirmed histologically. Ten of these patients had previously had total colitis, as shown by colonoscopic examination, the others had previously had colitis affecting the transverse colon to rectum.

The colitis in all these patients was clinically in remission. None were systemically unwell or had diarrhoea, blood in the stool or abdominal pain. The endoscopic appearances were normal or

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exhibited only hyperaemia and granularity, these areas being avoided when biopsy specimens were taken.

Triplicate biopsy specimens were obtained from each of five sites of the large bowel; ascending colon (ileoocaecal valve to hepatic flexure), transverse colon (hepatic to splenic flexure), descending colon (splenic to sigmoid flexure), sigmoid colon (sigmoid flexure to proximal rectal valve), and rectum.

Control mucosal biopsy specimens were obtained from the above five regions of the large bowel in 28 patients who had normal mucosa (10 male, median age 65, range 27–83 years). Fourteen of these patients had a normal colonoscopy for changed bowel habit, three a surveillance colonoscopy for polyps with no signs of recurrence, and four a surveillance colonoscopy for carcinoma with no signs of recurrence. Mucosal biopsy specimens were taken from a further seven patients under direct vision at the time of laparotomy for malignancy. These specimens were taken at least 15 cm away from any area of macroscopic abnormality, ensuring no cytochemical abnormality.¹⁰ All the patients in both groups had had a standard bowel preparation regimen using picolax (sodium picosulphate 10 mg, magnesium oxide 3.5 g, and citric acid 12 g supplied by Ferring Pharmaceuticals, Feltham, Middlesex UK). None of the patients were using steroids or any topical preparation, however, of those with ulcerative colitis all were taking a 5 aminosalicylic acid type of drug and three were also taking azathioprine.

DETERMINATION OF METABOLIC ACTIVITY

The metabolic capability of each biopsy specimen was determined by measuring the ability of the sample to oxidise ¹⁴C-labelled glucose, glutamine or butyrate to ¹⁴C-carbon dioxide over a two hour incubation period. Details of this assay and its validation have been published previously.⁸ Briefly, a biopsy specimen (5–10 mg wet weight) was blotted dry and placed in a 20 ml McCartney bottle that contained 2 ml of sterile RPMI 1640 cell culture medium (Gibco Life Technologies Ltd, Paisley, Scotland) supplemented with 5 mM L-glutamine and 5 mM sodium butyrate, 11.11 mM glucose was already present: the pH was re-adjusted to 7.4 by titrating with 1 M sodium hydroxide.

Glucose (Amersham) (148 KBq D-[U-¹⁴C]) or 37 KBq L-[U-¹⁴C] glutamine (Amersham) or 3.7

KBq [1-¹⁴C] sodium butyrate (New England Nuclear) was added to each incubation bottle to trace the conversion of these substrates to carbon dioxide. The bottle was incubated in a shaking water bath for two hours at 37°C in a 100% oxygen environment. Sulphuric acid (0.5 ml of 1 M) was added to terminate the incubation and displace the dissolved carbon dioxide from solution. This was absorbed by sodium hydroxide soaked onto filter paper. The quantity of trapped radioactivity was assessed by liquid scintillation counting. The biopsy specimens were recovered and their protein content estimated by the method of Lowry *et al.*¹¹ This allowed the rate of oxidation of fuel substrate to carbon dioxide to be expressed in pmol/μg of mucosal protein/hour.

HISTOLOGICAL ASSESSMENT OF BIOPSY SPECIMENS
A further biopsy specimen was taken from each of the ulcerative colitic patients for histological confirmation of the diagnosis. Similarly, control biopsy specimens were obtained from 14 patients who had normal colonic mucosa.

Quantification of the chronic inflammatory cell infiltrate (lymphocytes, plasma cells, eosinophils, and mast cells) in the lamina propria of the biopsy specimens was performed to determine if there was a numerical difference in the colitic patients and normal mucosal cell population. The specimens had been fixed in 10% formalin, embedded in paraffin wax, and stained with haematoxylin and eosin. The number of inflammatory cells were counted in 10 high power fields and a mean calculated for each specimen.

ETHICAL COMMITTEE APPROVAL

This study was approved by the Tower Hamlets District Ethical Committee and full informed consent was obtained from each patient before the biopsy specimens were taken.

STATISTICAL ANALYSIS

No assumptions were made about the distribution of the data so the Mann-Whitney U and Kruskal-Wallis H non-parametric tests were used to assess statistical significance. CIA software (BMA Publications, Tavistock Square, London, UK) was used to calculate the confidence intervals using the Wilcoxon method.

CALCULATIONS

The efficiency of the scintillation counter was established by using known concentrations of [U-¹⁴C]-glucose, placed into a solution of similar quench characteristics to the samples, thus the counts per minute could be converted to disintegrations per minute. The specific activity of each fuel substrate was calculated from the radioactive concentration given by the manufacturer's data sheet, allowing for the dilution that occurred when it was added to the unlabelled substrate in the cell culture medium. The quantity of fuel oxidised to carbon dioxide was equal to the number of disintegrations per second divided by the specific activity.

TABLE I Regional rate of mucosal oxidation of glucose, glutamine, and butyrate to carbon dioxide in ulcerative colitis

	Glucose	Glutamine	Butyrate
Ascending colon	8.26 (9) 5.98 to 12.83	40.3 (10) 28.8 to 116	324 (11) 149 to 844
Transverse colon	6.22 (13) 3.81 to 13.11	28.2 (14) 18.8 to 86.5	319 (12) 194 to 538
Descending colon	6.46 (14) 4.70 to 11.0	29.9 (15) 18.4 to 52.0	263 (15) 177 to 439
Sigmoid colon	7.43 (12) 4.40 to 13.4	41.7 (13) 25.9 to 68.6	318 (13) 215 to 479
Rectum	11.6 (12) 7.92 to 18.4	58.3 (13) 32.6 to 97.1	317 (13) 189 to 698
H	5.2*	4.7*	1.9*

Units: pmol/μg protein/hour; median and 95% confidence intervals (no of patients studied); Kruskal-Wallis H test
* = p > 0.05.

TABLE II Rate of oxidation of glucose, glutamine, and butyrate to carbon dioxide in normal and ulcerative colitic mucosa

	Normal	Ulcerative colitis	U
Glucose	8.75 (24) 6.74 to 11.1	7.15 (15) 5.25 to 15.1	164*
Glutamine	37.8 (27) 24.9 to 61.6	32.8 (15) 23.5 to 62.2	198*
Butyrate	472 (23) 351 to 637	272 (15) 199 to 368	92**

Units: pmol/ μ g protein/hour; median and 95% confidence intervals (no of patients studied); Mann-Whitney U test * $p > 0.05$, ** $p = 0.016$.

Results

ASSESSMENT OF REGIONAL VARIATION IN ULCERATIVE COLITIS

While there was a degree of variation between the colonic utilisation of the three substrates among subjects, there was no significant regional difference in the rate of fuel oxidation (Kruskal-Wallis $H = 5.2, 4.7, 0.82$ for glucose, glutamine and butyrate respectively $p > 0.05$, Table I).

As there was no regional variation in the rate of substrate consumption, a mean value was calculated from the five biopsy specimens from each patient. Thus the median and 95% confidence intervals for the population were obtained (Table II).

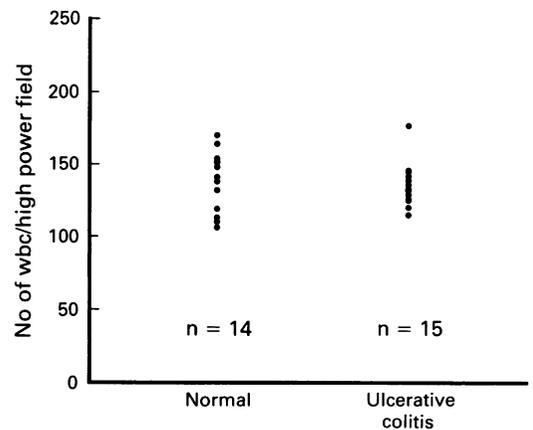
It has previously been reported, using this technique, that there was no significant regional variation in the utilisation of the three fuel substrates in healthy colonic mucosa⁸ and this was confirmed in this study (Table III Kruskal-Wallis $H = 1.7, 8.9, 1.9$ for glucose, glutamine and butyrate respectively $p > 0.05$). Thus, similarly, it was possible to calculate a median and 95% confidence intervals for the normal population (Table II) with results from all sites of the large bowel. These results confirm that butyrate is the preferred fuel source for normal and colitic large bowel mucosa followed by glutamine (Mann-Whitney $U = 47$ $p < 0.01$, $U = 8$ $p < 0.01$ respectively) and then glucose (Mann-Whitney $U = 30$ $p < 0.01$, $U = 18$ $p < 0.01$ respectively).

Only one attempt was made to obtain each sample to minimise the duration of colonoscopy hence values were not obtained for all of the colonic regions. Rarely (<2%), results were not obtained either because of contamination of the

TABLE III Regional rate of mucosal oxidation of glucose, glutamine, and butyrate to carbon dioxide in normal large bowel

	Glucose	Glutamine	Butyrate
Ascending colon	7.96 (9) 6.0 to 12.8	43.1 (10) 29.2 to 453	458 (9) 312 to 1490
Transverse colon	7.0 (10) 4.9 to 17.2	65.0 (11) 42.8 to 428	469 (11) 312 to 1294
Descending colon	10.2 (11) 6.7 to 19.8	40.5 (11) 23.8 to 491	611 (10) 406 to 4460
Sigmoid colon	7.9 (22) 7.3 to 12.8	22.2 (23) 20.2 to 60.5	388 (20) 334 to 629
Rectum	10.1 (12) 6.3 to 14.8	83.8 (12) 33.5 to 219.5	489 (11) 362 to 684
H	1.7*	8.9*	1.9*

Units: pmol/ μ g protein/hour; median and 95% confidence intervals (no of patients studied); Kruskal-Wallis H test * $p > 0.05$.



Chronic inflammatory cell infiltrate in normal and colitic mucosal biopsy specimens. Units: number of white blood cells (wbc)/high power field. Mann-Whitney $U = 120$ $p > 0.05$.

filter paper with neat isotope or failure to retrieve the specimen for protein estimation.

RATE OF FUEL OXIDATION IN COLITIC AND HEALTHY MUCOSA

There was no significant difference between the rate of glucose and glutamine oxidation in normal and colitic large bowel mucosa. The mucosal biopsy specimens, however, from sufferers of quiescent ulcerative colitis showed a significantly impaired ability to oxidise butyrate ($p = 0.016$) when compared with healthy large bowel mucosal biopsy specimens. Although the patients in the control group were significantly older than those in the colitic group (Mann-Whitney $U = 140$ $p = 0.04$), we have found no correlation between age and rate of substrate oxidation (data not shown).

HISTOLOGICAL EXAMINATION OF THE MUCOSAL BIOPSY SPECIMENS

Histological examination of the specimens confirmed the diagnosis of quiescent ulcerative colitis. There was no significant difference in the numbers of chronic inflammatory cells present in the specimens taken from colitic and normal large bowel mucosa (Mann-Whitney test $U = 120$ $p > 0.05$, Figure).

Discussion

Studies on human large bowel mucosal metabolism are rare, probably because of the difficulty in isolating human colonocytes and the need to use resection specimens, which are rarely completely normal. This technique, which uses biopsy samples, allows the rate of fuel oxidation to carbon dioxide to be studied quantitatively.

We have shown that colitic mucosa is able to utilise more butyrate than glutamine or glucose. This is in keeping with published results.⁶ We have been unable to show a regional difference in the utilisation of the three fuel substrates in healthy⁸ or quiescent colitic mucosa. Our finding of an impaired ability of quiescent colitic mucosa to oxidise butyrate supports Roediger's hypothesis that ulcerative colitis is a disease characterised by an energy deficiency.⁷ Roediger found the rate

of ^{14}C -carbon dioxide production from $[1-^{14}\text{C}]$ butyrate to be 72 pmol/hour/ μg dry weight in normal isolated colonocytes, decreasing to 36 pmol/hour- μg dry weight in those isolated from colitis patients. We report a similar percentage reduction in mucosal ability to oxidise butyrate, from 472 to 272 pmol/ μg mucosal protein/hour.

It is unlikely that this change in the metabolic potential of the mucosa is a consequence of inflammation because all the patients had quiescent or mild ulcerative colitis as assessed by their symptoms, mucosal endoscopic and histological appearance. That there is no significant difference in the numbers of chronic inflammatory cells makes it unlikely that this decrease in butyrate oxidation is a consequence of changes in the white cell population. Alternatively, it may be a result of the presence of 5-amino salicylic acid in the colitic group. We have shown, however, that the presence of this drug has no effect on the rate of butyrate oxidation in normal colonic mucosa.¹² We found no correlation between increasing age and increasing rate of butyrate oxidation making it unlikely that the younger age of the colitic group accounts for this decreased rate of butyrate utilisation.

In the rabbit, ketogenesis decreases from the caecum to rectum as more of the luminal butyrate is oxidised to carbon dioxide.¹³ There is evidence to suggest this is also true in the human large bowel and that the distal colon is more reliant on butyrate than the proximal colon. Our results did not show an increase in the flux of butyrate to carbon dioxide on progressing distally along the colon. This may have been because of the provision of alternative fuel sources (glucose and glutamine) in the incubation media. In vivo there would be little or no luminal glutamine in the distal colon, because it is readily utilised by the gut microflora.¹⁴

In ulcerative colitic colonocytes, Roediger reported an increase in the rate of glucose and glutamine utilisation, as measured by lactate and ammonia production respectively.⁷ We were unable to find a corresponding increase in the rate of carbon dioxide production from these two fuels. This may be because the glucose is accumulating as lactate rather than being oxidised by Krebs's cycle to carbon dioxide. It is known that in acute colitis there is an increase in faecal lactate.¹⁵ The rate of deamination of glutamine may be raised by increased flux through the ornithine-citrulline cycle resulting in greater ammonia production but not carbon dioxide.

Two clinical trials of butyrate, or short chain fatty acid enemata, in acute ulcerative colitis and one in non-specific proctosigmoiditis have resulted in clinical, endoscopic, and histological improvement.¹⁶⁻¹⁸ It has been suggested that the provision of exogenous butyrate, by creating

supraphysiological concentrations, may overcome the partial failure of butyrate oxidation in colitis by mass action.¹⁹ The patients in these three clinical studies, however, had severe colitis with diarrhoea and so initially may have had very low concentrations of luminal butyrate, which were replenished by the enemata. It may be that this comparative metabolic failure of butyrate oxidation is compounded in severe colitis by very low concentrations of luminal butyrate, explaining the efficacy of butyrate enemata in severe disease.

In conclusion, this study confirms that oxidation of butyrate, the major fuel source for colonic mucosa is significantly impaired in quiescent ulcerative colitis. Further work needs to be directed at investigating whether the defect lies in the transport of butyrate into cells or in its β oxidation.

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