Mucosal enzyme activity for butyrate oxidation; no defect in patients with ulcerative colitis

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Abstract

Background—Butyrate is an important energy source for the colon and its metabolism has been reported to be defective in ulcerative colitis. One mechanism for defective butyrate metabolism in patients with ulcerative colitis could be an enzyme deficiency in the β -oxidation pathway of butyrate.

Aims—This study was undertaken to measure the activity of each enzyme involved in the β -oxidation pathway of butyrate in colonic epithelium.

Patients—Patients with ulcerative colitis (n=33), Crohn's colitis (n=10), and control subjects with colorectal cancer or diverticular disease (n=73) were studied.

Methods—Analysis was carried out using fluorometric and spectrophotometric techniques on homogenised epithelial biopsy specimens.

Results—Significantly increased butyryl CoA dehydrogenase activity was found in mucosa from patients with ulcerative colitis (33.2 (28.3, 38.1) $\mu\text{mol/g wet weight/min:mean}$ (95% CI)) compared with activity in mucosa from control patients (24.3 (20.9, 27.7) $\mu\text{mol/g wet weight/min:mean}$ (95% CI)) $p < 0.02$. No significant increase in activity of the enzymes butyryl-CoA synthetase, crotonase or hydroxybutyryl-CoA dehydrogenase was found in patients with ulcerative colitis. In contrast the mucosal thiolase activity was significantly lower in those patients with quiescent colitis (3.21 (2.61, 3.81) $\mu\text{mol/g wet weight/min:mean}$ (95% CI)) when compared with control mucosa (5.69 (5.09, 6.29) $\mu\text{mol/g wet weight/min:mean}$ (95% CI)) $p < 0.001$. However, mucosal thiolase activity increases with the age of the donor patient and differences in the age range of the patient groups probably account for this finding.

Conclusions—This study shows no substantial deficiency of enzyme activity in the β -oxidation pathway of butyrate in the mucosa of patients with ulcerative colitis in histological remission.

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Roediger that metabolism of luminal butyrate may be abnormal in patients with ulcerative colitis and that this abnormality may result in energy deficiency at the cellular level,⁵ thus contributing to the colitis.

By using in vitro metabolic studies, Roediger showed that butyrate was the fuel of choice for colonic epithelial cells compared with alternative fuels such as glucose or glutamine.⁶ Roediger also found that when colonic cells were studied from patients with ulcerative colitis these cells oxidised butyrate as an energy source to a lesser extent than cells from control tissues.⁵ Furthermore the inability of colonic epithelial cells to utilise butyrate occurred irrespective of the activity of the colitis, suggesting that the abnormality might represent a primary defect in the mucosa and not be secondary to the inflammation in the colonic tissue.

Ireland and Jewell⁷ and Chapman *et al*⁴ confirmed that utilisation of butyrate is decreased in colonic cells from patients with quiescent ulcerative colitis. But Chapman *et al* were unable to confirm an earlier finding by Roediger² that butyrate metabolism was greater in the descending colon compared with the ascending colon.⁸

Both groups found that 5-aminosalicylic acid had no effect on butyrate metabolism in colonic epithelial cells from normal controls.^{7,9}

In contrast Finnie *et al*,³ and Clausen *et al*¹⁰ were unable to demonstrate any defect of butyrate metabolism in mucosal biopsy specimens from patients with quiescent colitis compared with mucosa from normal controls. These authors suggested that the discrepancy with previous studies may have arisen either from the consequences of the epithelial cell isolation technique or because of the use of potentially toxic concentrations of butyrate.³

It is possible that abnormal butyrate oxidation may occur in the colonic epithelial cells of patients with quiescent ulcerative colitis. Such an abnormality may be of pathogenetic significance and therefore requires further study. This study set out to measure the activity of each enzyme involved in oxidation of butyrate (Fig 1).

Methods

Tissue samples

Prior to assay all tissue samples were coded so that the biochemist performing the assays was unaware of the disease group of the donor patient. It was not possible to assay all enzymes in the oxidation pathway in each

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There is evidence that a metabolic defect within colonic epithelial cells could contribute to the pathogenesis of ulcerative colitis. Colonic epithelial cells absorb luminal butyrate and oxidise it to provide energy through the citric acid cycle.¹⁻⁴ It was proposed by

Figure 1: Pathway of β -oxidation of butyrate.

mucosal specimen. Therefore as many enzymes as possible were assayed depending on the amount of mucosa available for homogenisation from each patient. The effect of age, sex, or site of mucosa along the colon, or method of harvesting was assessed using normal mucosa.

Sample preparation

Samples of colorectal mucosa were transported to the laboratory in ice cold saline (0.15 M NaCl). Samples were examined and excess moisture removed by blotting. Samples not for immediate analysis were snap frozen and stored at -40°C . Lamina propria lymphocytes were isolated and stored according to the method of Sachdev *et al.*¹¹ Lymphocytes were thawed slowly at room temperature and enzymes assessed similarly to whole tissue samples.

Mucosa was removed from the bowel wall by scraping with the edge of a clean microscope slide and then weighed. Biopsy specimens were weighed and disrupted in entirety. Previously isolated lamina propria lymphocytes were weighed and then homogenised. All cells were homogenised in enzyme extraction buffers at a ratio of 100 mg/ml (wet weight/volume) by using an Omni 5000 hand held homogeniser at 4°C .

Subjects

Mucosal specimens were taken from 73 subjects undergoing investigation or treatment for colorectal cancer ($n=69$) or diverticular disease ($n=4$). The median age of these patients was 67

(range 28–91) years and 39 were male. All mucosal specimens in these control patients were taken from a site at least 10 cm from any macroscopic abnormality and adjacent mucosal samples were histologically normal. Sixty of these mucosal specimens were taken at laparotomy and 13 at rigid sigmoidoscopy.

Mucosal specimens were taken from 33 subjects with ulcerative proctocolitis, median age 46 (range 18–83) years, 18 were men. Adjacent mucosal specimens were taken in each case for histological examination and assessment of severity of ulcerative colitis. Mucosal specimens were taken from 13 patients at laparotomy and 20 at rigid sigmoidoscopy.

Mucosal specimens were taken from 10 subjects with Crohn's colitis, median age 34 (21–67) years, two were men. All of these mucosal specimens were taken at laparotomy. All of these patients had coexisting small bowel Crohn's disease. The diagnosis of ulcerative or Crohn's colitis was established using conventional clinical, radiological, endoscopic, and histological criteria.

Enzyme extraction

Butyryl-CoA dehydrogenase and hydroxybutyryl-CoA dehydrogenase – mucosal homogenates were extracted into 50 mM phosphate buffer (pH=7.0) containing 0.3 mM EDTA, 200 μM phenylmethylsulphonyl fluoride, and 2 mM dithiothreitol. Crude homogenate was reserved for hydroxybutyryl-CoA dehydrogenase assay. Homogenate for butyryl-CoA dehydrogenase assay was fractionated by addition of solid ammonium sulphate to 40% w/v with mixing for 30 minutes on ice and then centrifugation at $5000\times g$ at 4°C for 15 minutes. The supernatant was assayed.

Crotonase, acetoacetyl-CoA thiolase, and butyryl-CoA synthetase were extracted into 10 mM TRIS buffer (pH=7.4) containing 1 mM EDTA, 3 mM magnesium chloride, and 300 mM sucrose.

Cytological assessment of cell suspensions

Before complete disruption by homogenisation, aliquots of mucosal cell suspensions from the normal colon of three patients were spread on glass slides and sprayed with polyethylene glycol, ethyl alcohol, and glacial acetic acid fixative and stained with Papanicolaou stain. The proportions of lymphocytes and epithelial cells in these preparations were then estimated microscopically for 10 high power ($\times 400$) fields.

Classification of mucosal samples from patients with ulcerative proctocolitis

Routine haematoxylin and eosin stained paraffin wax sections were prepared from samples of mucosa adjacent to those used for assay. Inflammatory changes were classified using the criteria of Truelove and Richards¹² (Appendix). Epithelial cell density was assessed using the grading system described by Goodman *et al.*¹³

TABLE I Enzyme activity in normal biopsy specimens. Enzyme is expressed in $\mu\text{mol/g}$ wet weight/minute. Numbers of samples and 95% confidence limits are also shown

Enzyme	Men	Women
Butyryl-CoA synthetase		
Mean	0.058	0.049
Number	4	9
95% CI	-0.062 to 0.178	0.019 to 0.079
Butyryl-CoA dehydrogenase		
Mean	21.97	25.54
Number	6	12
95% CI	14.77 to 29.2	20.7 to 30.38
Crotonase		
Mean	9.26	9.25
Number	16	19
95% CI	7.2 to 11.3	6.52 to 11.98
Hydroxybutyryl-CoA dehydrogenase		
Mean	4.24	4.59
Number	34	30
95% CI	3.79 to 4.69	4.1 to 5.08
Thiolase		
Mean	5.96	5.42
Number	34	31
95% CI	5.27 to 6.65	4.4 to 6.44

Enzyme assays

All chemicals and enzymes were purchased from Sigma Chemical Company UK. Assays were performed either spectrophotometrically using a Philips PU8720 spectrophotometer or fluorimetrically using a Shimadzu RF-5001PC fluorimeter.

All enzyme assays were optimised for human colonic epithelial cells.

Butyryl-CoA synthetase (BuCoAs) was assayed according to the method of Lageweg *et al.*¹⁴ This enzyme catalyses the activation of butyrate to its CoA ester. The reaction was assessed by coupling the reaction to acyl-CoA oxidase and peroxidase. The rate of peroxide formation was assessed fluorimetrically. The intra-assay coefficient of variation was 9.6%.

Butyryl-CoA dehydrogenase (BuCoADH) was assayed according to the method of Engel.¹⁵ BuCoADH catalyses the conversion of butyryl-CoA to crotonyl-CoA. The reaction

TABLE II Enzyme activity in $\mu\text{mol/wet weight/min}$ for control subjects in samples collected from rectal biopsy and surgical specimens from rectum, left colon, and right colon

Enzyme	Biopsy specimen (rectum)	Surgical specimen		
		Rectum	Left colon	Right colon
Synthetase	0.0783		0.0375	0.0251
BuCoA DH	23.41		32.24	21.50
Crotonase	8.58	7.90	8.45	13.26
HoBuCoADH	4.19	4.53	4.16	4.71
Thiolase	4.13*	6.14	5.44	6.75
Protein	3.71	4.22	3.62	3.53

Statistical analysis of $\log_{10}$ transformed data shows no significant differences from synthetase, butyryl-CoA dehydrogenase, crotonase and hydroxybutyryl-CoA dehydrogenase. Thiolase activity, however, showed significant differences (* $p=0.046$) between rectal biopsy samples and surgical samples. All subsequent statistical analyses of thiolase activity were performed on like samples.

was assessed by coupling this reaction to dichlorophenolindophenol and phenazine methosulphate. The rate of decreasing absorbance was assessed spectrophotometrically at 600 nM.

Crotonase was assayed according to the method of Fong and Schultz.¹⁶ Crotonase catalyses the conversion of crotonyl-CoA to hydroxybutyryl-CoA. The formation of the magnesium complex was assessed spectrophotometrically at 280 nm. The intra-assay and inter-assay coefficients of variation were 4% and 13% respectively.

Hydroxybutyryl-CoA dehydrogenase (Hobu-CoADH) activity was measured according to the method of Fong and Schulz.¹⁷ HobuCoADH catalyses the conversion of hydroxybutyryl-CoA to acetoacetyl-CoA. However, in vitro the forward reaction rapidly equilibrates thus the reaction was assayed in the reverse direction. The rate of reaction resulting from nicotinamide adenine dinucleotide (NAD) formation was assessed spectrophotometrically at 340 nm. The intra-assay and inter-

Figure 2: Comparison of enzyme activities between control subjects and ulcerative colitis patients classified as Truelove and Richards class 1. Control values are shown as $\square$ and ulcerative colitis samples as $\bullet$. The mean enzyme activity is shown by horizontal line.

TABLE III Variation of enzyme activity with age for control and ulcerative colitis samples

Enzyme	30-50		51-70		71-90	
	Control	UC	Control	UC	Control	UC
Synthetase	0.03	0.032	0.052	0.034	0.067	0.021
BuCoA DH	29.31	28.84	27.25	24.31	18.91	25.45
Crotonase	6.63	8.95	10.76	11.37	7.97	7.63
HoBuCoA DH	4.51	3.85	4.33	3.13	4.66	2.96
Thiolase	3.43	3.69	5.74	2.37	6.12	5.84

assay coefficients of variation were 8.2% and 16.7% respectively.

Acetoacetyl-CoA thiolase (Thiolase) activity was assayed according to the method of Williamson *et al.*¹⁸ Thiolase catalyses the conversion of acetoacetyl-CoA to two acetyl-CoA units. The rate of reaction due to acetyl-CoA formation was assessed spectrophotometrically at 303 nm. The intra-assay coefficient of variation was 4%.

Protein concentration was determined using the method of Bradford.¹⁹ Protein concentrations in control samples, ulcerative colitis, and Crohn's samples were compared. No differences were seen between normal control samples and samples from patients with ulcerative colitis. Therefore no adjustment for protein concentration was made. In the case of Crohn's samples differences in protein

concentration with the control samples were observed and therefore all results were expressed per gram of protein.

Statistical methods

Comparisons in enzyme activity between groups of patients were assessed using an unpaired *t* test with two tail *p* values on log₁₀ transformations of the enzyme data. Other statistical tests are as indicated in the text.

Ethics

Approval for the study was given by the North Birmingham Research and Ethics Committees.

Results

Enzyme activity in histologically normal mucosa

Table I summarises enzyme activity in normal biopsy specimens. No differences in enzyme activity were found between men and women for any of the enzymes tested (two sample *t* test). One way analysis of variance showed no site related difference in enzyme activity between samples from the rectum, left or right colon. There was increased thiolase activity with age when patients were subdivided into three groups: those below 50 years, those between 50 and 70 years, and those over 70 years of age. Statistical analysis (one way analysis of variance) on log₁₀ transformed data showed significant differences in enzyme activity between groups ($F=4.83$; $p=0.01$). No other enzyme activity varied significantly with age. Thiolase activity also varied significantly between rectal biopsy and laparotomy samples with the biopsy specimens giving lower values (two sample *t* test $p=0.008$). Table II gives these results. All further comparisons were stratified into biopsy and rectal excision samples. No other enzyme activity varied with the mode of collection.

Protein

Fifty four specimens were assayed for protein content. The mean (95% CI) protein concentration was 3.79 (3.52, 4.06) g/g wet weight tissue. No differences were seen across the age range of patients tested (45-91 years). Comparative analysis by site of origin of sample showed no differences between rectum, left colon or right colon (Table II).

Enzyme activity in ulcerative colitis mucosa

Butyryl-CoA synthetase - 18 patients donated biopsy specimens, eight were male and 10 were female. The median age was 55 years and the age range spanned 20 to 80.

Butyryl-CoA dehydrogenase - 24 patients donated biopsy samples, 15 were male and nine were female. The median age was 39 years and the age range spanned 18 to 83.

Crotonase - 20 patients donated biopsy specimens, 13 were male and seven female.

Figure 3: Comparison of enzyme activity between Truelove and Richards classes. Butyryl-CoA synthetase (■), butyryl-CoA dehydrogenase (□), crotonase (▨), hydroxybutyryl-CoA dehydrogenase (▩), thiolase (▧).

The median age was 38 years and the age range spanned from 18 to 83.

Hydroxybutyryl-CoA dehydrogenase – 24 patients donated biopsy specimens, 13 were male and 11 female. The median age was 39 years and the age range spanned 18 to 83.

Thiolase – 20 patients donated biopsy samples, 13 were male and seven were female. The median age was 38 years and the age range spanned 18 to 72.

Comparison of enzyme activity in ulcerative colitic mucosa with histologically normal mucosa

For each enzyme studied a comparison was made between the enzyme activity in control biopsy specimens and the enzyme in Truelove and Richards (T and R) class 1 biopsy specimens (two sample *t* test on $\log_{10}$ transformed data). The mean (95% CI) butyryl CoA dehydrogenase activity ($\mu\text{mol/g wet weight/min}$) in normal mucosa was 24.3 (20.9, 27.7) $n=18$ compared with 33.2 (28.3, 38.1) $n=10$ in biopsy specimens of T and R class 1 ($p=0.02$). The mean thiolase activity was 5.69 (5.09, 6.29) $n=65$ in normal mucosa compared with 3.21 (2.61, 3.81) $n=9$ in samples of T and R class 1 ($p=0.001$). There was no significant difference in butyryl-CoA

synthetase, crotonase, or hydroxybutyryl-CoA dehydrogenase activity between control and T and R class 1 biopsy specimens (Fig 2).

The mean thiolase activity in T and R class 1 biopsy specimens was significantly lower than the mean thiolase activity in control samples. Within the T and R class 1 biopsy specimens the median age of the donor patients was 37 (range 28–62) years compared with a median age of 67 (range 28–85) years in patients with normal mucosa. In view of this the youngest nine control patients: median age 49 (range 28–52) years were selected from the control group and thiolase activity in these biopsy specimens was compared with the thiolase activity in the T and R class 1 samples. No significant difference was found (two sample *t* test on $\log_{10}$ transformed data $p=0.6$). Table III shows these data.

Differences in enzyme activity in Truelove and Richard classes

Enzyme activity was compared between each of the three Truelove and Richards classes (one way analysis of variance on $\log_{10}$ transformed data). Butyryl-CoA dehydrogenase activity was significantly different between T and R classes. Hydroxybutyryl-CoA dehydrogenase activity was also significantly different between T and R classes with enzyme activity falling with increasing inflammation in the adjacent mucosal samples (Fig 3).

Correlation of enzyme activity with epithelial cell density

Biopsy specimens from patients with ulcerative colitis were classified according to their epithelial cell density. There was a trend towards decreasing enzyme activity with decreasing cell density, but between the grades 1, 2, 3 there was no significant difference (Fig 4). No specimens were classified as class 4.

Correlation of enzyme activity with drugs taken by patient

The mean enzyme activity in ulcerative colitis patients taking either oral or intravenous corticosteroids ($n=8$) was compared with the enzyme activity in those patients taking neither topical nor systemic corticosteroids ($n=9$). An unpaired *t* test on the $\log_{10}$ transformed data showed no significant difference in any enzyme activity.

A similar analysis comparing the enzyme activity in patients taking any 5-aminosalicylic containing drug orally or rectally ($n=17$) with the enzyme activity in those patients taking no 5-aminosalicylic containing drug either orally or rectally ($n=5$) showed no significant differences.

Enzyme activity in the mucosa of patients with colonic Crohn's disease

Butyryl-CoA dehydrogenase – samples from nine patients were assayed for butyryl-CoA dehydrogenase activity. The mean activity was 1.33 (0.83, 1.83) $\mu\text{mol/g protein/min}$.

Figure 4: Comparison of enzyme activity in tissue classified according to epithelial cell density. Butyryl-CoA synthetase (■), butyryl-CoA dehydrogenase (□), crotonase (▨), hydroxybutyryl-CoA dehydrogenase (▩), thiolase (▤). H = hypertrophied crypt.

Figure 5: Comparison of enzyme activity in Crohn's and control tissue. The enzyme activity is expressed in $\mu\text{mol/g protein/minute}$. Crohn's disease (▨), control (■). * $p < 0.05$ unpaired t test on $\log_{10}$ transformed data.

Crotonase – samples from nine patients were assayed for crotonase activity. The mean activity was 0.41 ($0.25, 0.57$) $\mu\text{mol/g protein/min}$.

Hydroxybutyryl-CoA dehydrogenase – 10 patients' samples were assayed for hydroxybutyryl-CoA dehydrogenase activity. The mean enzyme activity was 0.3 ($0.22, 0.38$) $\mu\text{mol/g protein/min}$.

Thiolase – samples from 10 patients were assayed for thiolase activity. The mean activity was 0.21 ($0.09, 0.33$) $\mu\text{mol/g protein/min}$.

Comparison of Crohn's and control tissue

Comparison of Crohn's and control tissue showed similar activities in the enzymes butyryl-CoA dehydrogenase and thiolase. In the case of crotonase and hydroxybutyryl-CoA dehydrogenase enzyme activities were significantly higher in Crohn's tissue than the controls (Fig 5) (unpaired t test on $\log_{10}$ transformed data).

Lamina propria lymphocytes

Homogenates of lamina propria lymphocyte prepared from normal, ulcerative colitis, and Crohn's colonic tissue were assayed for a range of enzyme activities. No butyryl-CoA synthetase activity was measurable in lamina

propria lymphocytes from control, ulcerative colitis, or Crohn's disease patients. Measurable enzyme activity was seen in each tissue type for the enzymes butyryl-CoA dehydrogenase, Crotonase, hydroxybutyryl-CoA dehydrogenase, and thiolase (Fig 6). No differences in enzyme activities were seen between the tissue types except that higher hydroxybutyryl-CoA dehydrogenase activity was seen in ulcerative colitis lymphocytes. Cytological studies show that less than 10% of cells in the homogenate were lamina propria lymphocytes the remaining 90% being colonic epithelial cells.

Discussion

This study represents the first attempt to measure the activity of the enzymes responsible for the oxidation of butyrate in human colonic epithelial tissue. All enzymes were detected and measured although the dehydrogenation step mediated by hydroxybutyryl-CoA dehydrogenase equilibrates so rapidly in vitro that it requires assay in the reverse direction. One unexpected finding was an increase in thiolase activity with increasing age. The mechanism for this phenomenon remains unclear. The increase in thiolase activity with increasing age might explain the apparent difference in thiolase activity between control and colitic tissue. No other important variations of enzyme activity within normal colon were noted with age, sex, or position around the colon. There was a significantly lower thiolase activity in endoscopic biopsy specimens compared with operative specimens.

In ulcerative colitis each of the enzymes butyryl-CoA synthetase, butyryl-CoA dehydrogenase crotonase, and hydroxybutyryl-CoA dehydrogenase showed increased activity in Truelove and Richards class 1 mucosa compared with that found in control mucosa. But only for butyryl-CoA dehydrogenase did this rise reach significance. There was an inverse relation between mucosal inflammation and enzyme activity. In the most severely affected mucosa (Truelove and Richards 3) there was a tendency for activity to increase once again. For butyryl-CoA and hydroxybutyryl-CoA dehydrogenases these changes reach significance. One possible explanation for this finding is that although there is probably a generalised depression in enzyme activity with increasing damage to epithelial cells by the inflammatory process, this depression may be offset by increased crypt cell production. Crypt cell production is known to increase with increasing disease activity in the mucosa of patients with ulcerative colitis.²⁰ Thus enzyme activity in each particular Truelove and Richards class may depend on a balance between inflammation and cell production.

For thiolase activity, there was a different change in enzyme activity with a significant fall in activity in Truelove and Richards class 1 compared with control biopsy specimens. This at first sight could be interpreted as an abnormality with potential significance for the pathogenesis of ulcerative colitis. Further assessment shows that this finding is almost

Figure 6: Enzyme activities in lamina propria lymphocytes from control (stippled), ulcerative colitis (white), and Crohn's disease (hatched) tissues. Enzyme activity is expressed as $\mu\text{mol/g wet weight tissue/min}$.

certainly due to the increased age of the control group, with correspondingly increased enzyme activity, compared with the younger age of the colitic group. Furthermore thiolase activity is required to catalyse the final step in the oxidation of butyrate and it is probable that butyryl-CoA synthetase controls the rate limiting step of the oxidation pathway.

This study therefore shows a rise in enzyme activity in minimally inflamed mucosa from patients with ulcerative colitis compared with normal mucosa.

There was a tendency for all enzyme activity studied to fall in parallel with falling epithelial cell density in the mucosa of patients with ulcerative colitis. This type of relation was previously described as a property of the colonic epithelial cell enzyme glucosamine synthetase.¹³ This is probably a generalised response to epithelial cell depletion and may be shared by many colonic epithelial cell enzymes in patients with ulcerative colitis.

Previous studies show that 5-aminosalicylic acid has no effect on butyrate metabolism in colonic epithelial cells from normal controls. This study complements this finding by suggesting that neither corticosteroid nor 5-aminosalicylic acid treatment have any effect on the enzyme activity of the oxidation pathway of butyrate.

Enzyme activity in colonic mucosa from patients with Crohn's colitis was raised compared with that from the mucosa of control subjects. This rise in activity was significant for the enzymes butyryl-CoA dehydrogenase and thiolase. This rise in activity mirrors the increased activity seen in Truelove and Richards class 1 mucosa from patients with ulcerative colitis. It probably occurred because of selection of the least inflamed tissue in the colon for assay. Previous studies of the enzyme glucosamine synthetase¹³ report a rise in the activity of this enzyme in the mucosa of patients with Crohn's colitis even at sites distant from the macroscopically evident disease. The rise in enzyme activity found in mucosa from patients with Crohn's colitis is probably not caused by an increased cell turnover in the Crohn's tissue because cell proliferation in rectal biopsy specimens from patients with rectal Crohn's disease is normal irrespective of the degree of inflammation in the rectum.²¹ It seems more probable that the disease process triggers a generalised reactive increase in the activity of many intracellular enzymes. This finding suggests that the observed rise in glucosamine synthetase activity in patients with Crohn's colitis is part of a more widespread response to inflammation in the colonic epithelial cell.

This study used biopsy specimens and not epithelial cell preparations to study colonic epithelial cell enzyme activity and therefore consideration must be given to sources of enzyme activity within the samples but not arising from the colonic epithelial cells themselves. Although colonic wall muscle does not metabolise butyrate³ the mucosal homogenate used for enzyme assay contained a mixture of both colonic epithelial cells and lamina propria lymphocytes. Cytological assessment of these cell mixtures suggested that lamina propria lymphocytes accounted for only 10% of the cells in the mixture. Further studies on isolated lamina propria lymphocytes showed a small but detectable enzyme activity from these cells. This activity was similar irrespective of whether the cells were from control subjects or patients with inflammatory bowel disease. They are therefore likely to have made a minimal but relatively constant contribution to the total enzyme activity in the homogenates irrespective of the source of the specimens.

In conclusion, this study suggests that if abnormalities of butyrate metabolism occur in the colonic epithelium of patients with ulcerative colitis then the mechanism of this abnormality does not entail decreased enzyme activity in the β -oxidation pathway for butyrate. None the less an abnormality of butyrate metabolism in patients with ulcerative colitis remains possible. It seems unlikely that supply of butyrate to colonic epithelial cells is ever in danger because studies of colonic luminal bacterial flora in patients with ulcerative colitis show few changes.²² In addition absorption of butyrate in the remission phase of the disease is no different from absorption in control subjects.²³ However, other abnormalities of intracellular butyrate

metabolism may occur such as abnormal intracellular transport of butyric acid, alteration of mitochondrial oxidation or abnormal incorporation of butyrate products into the citric acid cycle for energy production.

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Appendix Summary of Truelove and Richards classification for histological severity of ulcerative colitis

No significant inflammation	
Inflammation	Nil
Crypt abscesses	Absent
Epithelium	Intact
Gland architecture	Glands reduced in number Stunted and atrophic
Mild to moderate inflammation	
Inflammation	Increased chronic inflammatory cell Mild increase in neutrophils Variable intensity
Crypt abscesses	Occasional
Epithelium	Increased proliferation Intact (apart from crypt abscess)
Gland architecture	Irregular shape and density
Severe inflammation	
Inflammation	Chronic inflammatory cell infiltrate
Crypt abscesses	Plentiful + erosions of crypt epithelium Inflammation extending into lamina propria
Epithelium	Small breaches and erosions Irregular and often cuboid cells adjacent to erosions
Gland architecture	Markedly irregular in density and shape with gland destruction