Ileal and colonic epithelial metabolism in quiescent ulcerative colitis: increased glutamine metabolism in distal colon but no defect in butyrate metabolism

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Abstract

Previous studies have shown that butyrate is an important energy source for the distal colon, and that its metabolism may be defective in ulcerative colitis (UC). A similar metabolic defect in the ileum might account for the occurrence of 'pouchitis' in UC patients after colectomy. A method has been developed that allows the measurement of metabolism in ileocolonoscopy biopsy specimens, and this has been used to assess butyrate and glutamine metabolism in quiescent UC and controls. Preliminary experiments showed optimal metabolism of butyrate at 1 mmol/l. In controls glutamine metabolism was greater in the ascending (mean (SD)) (4.9 (3.2) nmol/h/ μ g protein) than in the descending colon (1.4 (0.7)) ($p < 0.05$, Mann-Whitney U test), but butyrate metabolism was similar in the two regions (ascending 62.6 (44.2), descending 51.5 (32.0)). Consequently ratios of butyrate/glutamine metabolism were higher in the descending colon (20.6 (14.3)) than in the ascending colon (14.3 (9.6)) ($p < 0.05$). In UC, rates of butyrate metabolism were similar in the ascending (92.5 (58.3) nmol/h/ μ g protein) and descending (93.3 (115)) colon, and these were not significantly different from controls. In UC, glutamine metabolism was similar in the ascending (6.2 (7.7) nmol/h/ μ g protein) and descending colon (7.8 (7.9)); the metabolism in the descending colon was significantly greater than in controls ($p < 0.01$). Butyrate (135 (56) nmol/h/ μ g protein) and glutamine (24.1 (16.2)) metabolism in the ileum in UC, were not significantly different from control values (butyrate 111 (57), glutamine 15.5 (15.6)). These results confirm that there is regional variation of nutrient utilisation throughout the colon, but they do not support the hypothesis that UC is caused by a deficiency of butyrate metabolism.

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There is strong evidence that colitis can result from impaired mucosal metabolism. It has been shown that the colonic epithelium obtains much of its energy supply from fatty acids, particularly butyrate, which are present in the colonic lumen as a result of fermentation of carbohydrates by bacteria. Colitis occurs in the defunctioned colon after diversion of the faecal flow and subsides when the faecal flow is replaced.^{1,2} The occurrence of colitis in animals that are vitamin deficient³ or that are treated with inhibitors of fatty acid metabolism⁴ supports the theory that fatty acid metabolism is vital to the well being

of the colonic epithelium. Colitis, which is histologically similar to ulcerative colitis, has also been described in association with pellagra.⁵

How the various forms of nutritional colitis relate to ulcerative colitis (UC) is uncertain, but studies have suggested that metabolism of the short chain fatty acid butyrate is impaired in UC.⁶⁻⁹ In these studies, in common with many others, it has not been possible to discover if the change was a primary defect or merely a secondary effect of the disease process.

The frequency of 'pouchitis' in ulcerative colitis patients who have colectomy and pouch construction contrasts with the apparent rarity of pouchitis in patients who have the same operation for familial polyposis.¹⁰ This implies a potential abnormality of the ileal mucosa in ulcerative colitis that is not evident when the ileum and colon are in normal continuity, with the possible exception of 'backwash ileitis'. In view of this it seems appropriate to study mucosal metabolism in ileal as well as colonic mucosa in UC patients and controls in the expectation that any abnormalities that could be detected in the histologically normal ileum of ulcerative colitis patients would be unlikely to be secondary phenomena.

A further problem with the interpretation of some previous studies of mucosal metabolism^{6,7} has been concern that the procedure used for isolating a pure epithelial cell population from resected bowel specimens may itself have introduced artefacts, and that these artefactual changes might well have been different in the inflamed colitic tissue from those in the histologically normal colon.

This paper describes a technique that allows metabolism of nutrients to be assessed in whole epithelial biopsy specimens, and this technique has been used to assess the metabolism of butyrate and glutamine by ileal and colonic biopsy specimens from patients with idiopathic ulcerative colitis and controls.

Methods

MUCOSAL BIOPSY SPECIMENS

All specimens were taken using Olympus FB 13U colonoscopy forceps at routine colonoscopy, except in two patients in whom ileal specimens were obtained through an ileostomy with the same forceps. Bowel preparation in all colonoscopy patients was similar, consisting of PicoLax, two sachets on the day before colonoscopy and clear fluids for 48 hours. All patients gave written informed consent, and the studies were

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approved by the ethical committee of the Royal Liverpool University Hospital.

SUBJECTS

Colonic studies

Ascending and descending colonic biopsy specimens were taken from 12 patients with long-standing quiescent ulcerative colitis who were being examined as part of a programme to screen for dysplasia/malignancy, and from 12 patients who acted as controls, who did not have ulcerative colitis. The final diagnosis of the control patients was sporadic colonic polyps ($n=4$), diverticular disease (3), haemorrhoids (2), unexplained anaemia (1), carcinoma (1), and irritable bowel syndrome (1). All specimens in these control patients were taken from a site at least 5 cm from any macroscopic abnormality, and adjacent mucosal specimens, which were normal on histological examination were obtained in all.

The mean duration of colitis was 12 years (range 6–18). The median age of UC patients was 43 (28–65), which was not significantly different from the control median 48 (18–68) ($p=0.21$, Mann-Whitney U test). Sex ratios were similar, but none of the UC patients smoked whereas six controls were current smokers. All the UC patients were taking regular 5-amino salicylic acid preparations, but none was taking any form of corticosteroid.

Ileal studies

Terminal ileal biopsy specimens were taken at ileocolonoscopy (four patients) for patients with longstanding quiescent ulcerative colitis and by the ileostomy of two patients who had a colectomy for UC (histological reports state that 2 and 4 cm of ileum had been resected with the colectomy). Ileal specimens were taken from eight patients who did not have UC and who acted as controls. One of the UC patients who had had an ileostomy had mild non-specific inflammatory changes histologically (but no symptoms), but the ileal mucosa was histologically normal in all other cases. The mean duration of ulcerative colitis was 14 years, and these patients were having colonoscopy to exclude dysplasia/malignancy, except the patients with ileostomies who gave informed consent for ileoscopy specifically for this study. The median ages were similar (UC patients 48, controls 51). The final diagnosis in control patients was diverticular disease ($n=3$), colonic cancer (1), unexplained anaemia (1), and irritable bowel syndrome (3). Three controls smoked, none of the UC patients did so, and all UC patients except the two with ileostomies were taking regular 5-amino salicylic acid preparations.

MUCOSAL METABOLISM

The method that Veerkamp *et al*¹¹ devised to study fatty acid metabolism in skeletal muscle was adapted to study intestinal mucosal metabolism. After removal, mucosal biopsy specimens were placed in ice cold pre-gassed

Krebs-Henseleit buffer containing 11 mM glucose, and transported within 10 minutes to the laboratory. Each specimen was divided by scalpel into approximately 10 pieces per specimen (each piece approximately 1 mg), and then placed in a glass scintillation vial in 1 ml Krebs-Henseleit containing 11 mM glucose to which had been added either 1 μ Ci sodium [$1\text{-}^{14}\text{C}$] butyrate and 1 mM sodium butyrate, or 1 μ Ci L [^{14}C (U)] glutamine and 1 mM glutamine. An Eppendorf reaction tube was suspended above the culture medium, the vial was gassed with 95% O_2 /5% CO_2 and closed with a rubber seal (see Fig 1). The vials were cultured for two hours at 37°C in a shaking water bath (Grant Instruments, Cambridge, UK) set at 120 oscillations per minute. At the end of the culture period 0.25 ml 10% perchloric acid was injected into the culture medium to stop the reaction, and 0.5 ml of a solution of 66% ethane 1,2 diol/33% ethanolamine was injected into the Eppendorf tube to absorb $^{14}\text{CO}_2$. The vials were kept at 4°C for 90 minutes to allow equilibration, after which the Eppendorf reaction tubes were removed and placed in 10 ml scintillant, which was a mixture of 4 g/l Omnifluor in 2:1 toluene/methanol. After vigorous shaking, these were then counted in a Beckman scintillation counter. All counts were corrected by subtraction of counts for ^{14}C liberated in control vials without specimens.

Total biopsy specimens protein content was estimated at the end of the incubation period using a Lowry method¹² after ultrasonication (4 $\times$ 15 second bursts with an MSE (MSE Instruments, Crawley, UK) ultrasonicator at maximum power setting).

Results were expressed as nmol substrate metabolised per hour, after correcting for specific radioactivity of the substrate and number of ^{14}C atoms per molecule. For each patient, each assay was performed in duplicate at each site studied and the mean of the results calculated.

Comparisons of the metabolism between different sites in the same patient, and the same

Figure 1: Apparatus for measurement of metabolism by colonic biopsy specimens. For detailed description see methods section.

Figure 2: Rate of metabolism of butyrate by colonic biopsies. The rate of metabolism of ^{14}C butyrate to $^{14}\text{CO}_2$ by colonoscopic biopsy specimens taken from a resected colon was found to be linear for at least three hours. Metabolism is measured as number of nmol $^{14}\text{CO}_2$ produced from sodium $[1-^{14}\text{C}]$ butyrate per μg biopsy protein. Similar time course experiments for glutamine confirmed that rate of metabolism was linear for the duration of the culture period (results in text).

sites in UC patients and controls were analysed using a non-parametric (Mann-Whitney U) test. (95% confidence intervals (95% CI) for difference between means are quoted where appropriate.)

VALIDATION EXPERIMENTS

Small pieces (about 3×2 cm) of resected colon were obtained within 30 minutes of removal from patients having colectomy for cancer or diverticular disease, and placed in ice cold Krebs-Henseleit buffer at pH 7.4 (Sigma Chemical Co, Poole, UK) containing 11.1 mM glucose, gentamicin 100 $\mu\text{g}/\text{ml}$ and nystatin 60 $\mu\text{g}/\text{ml}$, which was pre-gassed with 95% $\text{O}_2/5\%$ CO_2 . The tissue studied was taken at least 5 cm distant from any macroscopic disease. The mucosal surface was then pinned out and biopsied with colonoscopy biopsy forceps. The specimens were then analysed using the method described previously for mucosal metabolism.

A series of experiments was performed to detect the optimal concentration of butyrate in the culture system. For these experiments specimens were cultured in the same concentration of radiolabelled sodium butyrate but with different concentrations of unlabelled sodium butyrate.

To assess the potential contribution of lamina

Figure 3: Metabolism of butyrate at different concentrations. Conversion of butyrate to CO_2 was greater at a concentration of 1 mM than at 10 mM ($p < 0.05$), or at 0.067 mM ($p < 0.01$), but did not differ significantly from 5 mM ($p = 0.33$) (Kruskal-Wallis).

propria lymphocytes to measured metabolism we obtained peripheral blood lymphocytes from the heparinised blood of a patient having colonoscopy for polyp surveillance with centrifugation in Lymphoprep (Flow Laboratories, Rickmansworth, UK). Lymphocytes (12.8×10^6) were prepared from 10 ml heparinised blood. Viability was measured using trypan blue exclusion and was found to be $>95\%$ both before and after two hours culture. Five 200 μl aliquots representing 2.56×10^6 lymphocytes were each placed in Krebs-Henseleit with a colonoscopic biopsy specimen taken from the same patient and metabolism measured as below for mucosal biopsy specimens. The results were compared with those obtained when culturing colonic specimens in the absence of the lymphocyte preparation.

Attempts were made to measure the relative contribution to the overall metabolism of mucosa, lamina propria, and muscularis layers. By gently scraping the mucosa and lamina propria from a small piece of resected colon with a scalpel blade and biopsying the tissue below, it was possible to compare metabolism of mucosal biopsy specimens with that of submucosal specimens (histologically shown to contain connective tissue and muscularis).

A further series of experiments was performed to assess whether colonic bacteria could contribute to measured metabolism. This was necessary in case there were different adherent bacteria on the mucosal surface in disease and controls, and for these experiments faecal fluid was aspirated at colonoscopy and added in 100 μl aliquots to a specimen in the culture system. Control experiments were performed adding 100 μl Krebs-Henseleit. In addition, experiments were conducted with, and without 100 $\mu\text{g}/\text{ml}$ gentamicin and 60 $\mu\text{g}/\text{ml}$ nystatin in the Krebs-Henseleit.

Results

VALIDATION EXPERIMENTS

The rates of metabolism of butyrate (Fig 2) and of glutamine were found to be roughly linear for at least three hours. For butyrate oxidation the rate in the first hour was (mean (SD)) 110.8 (16.5) nmol/ μg protein, over two hours 85.8 (11.1) nmol/h/ μg protein, and over three hours 90.3 (8.8) ($p < 0.0001$ for linearity test using grouped regression analysis (Arcus Pro-II)). For glutamine the rate of metabolism in the first hour was 5.1 (1.1) nmol/ μg protein, 4.6 (2.2) nmol/h/ μg protein over two hours, and 4.3 (1.0) over three hours ($p < 0.0001$ for linearity test). Metabolism of butyrate was optimal at 1 mM (Fig 3) and for this reason we decided to use 1 mM butyrate for the remainder of the study.

Reproducibility was checked with six biopsy specimens from adjacent sites in a single patient and coefficient of variation was found to be 12% for butyrate metabolism expressed per μg protein per hour and 18% for glutamine metabolism.

The mean overall coefficient of variation assessed by comparison of paired biopsy specimens was 20% for butyrate metabolism and 23% for glutamine metabolism.

TABLE I Metabolism of glutamine and butyrate by mucosal biopsy specimens

	Controls (n=12)	Ulcerative colitis (n=12)
Ascending colon butyrate	62.6 (44.2)	92.5 (58.3)
Ascending colon glutamine	4.9 (3.2)*	6.2 (7.7)
Ascending colon butyrate/glutamine	14.3 (9.6)†	14.6 (9.25)
Descending colon butyrate	51.5 (32)	93.3 (115)
Descending colon glutamine	1.4 (0.7)*‡	7.8 (7.9)‡
Descending colon butyrate/glutamine	20.6 (14.3)†	15.9 (15.6)

Mean figures for metabolism (nmol/μg protein/h) of each nutrient are given with standard deviation in brackets. Values with same superscript are significantly different at $p < 0.05$ (*) or $p < 0.01$ (‡) using Mann-Whitney U test.

Figure 4: Metabolism of butyrate (colon). Butyrate metabolism was similar in the ascending colon and descending colon of controls. Metabolism in ulcerative colitis patients did not differ significantly.

Figure 5: Metabolism of glutamine (colon). In controls, glutamine metabolism was significantly greater in the ascending than in the descending colon ($p < 0.05$, Mann-Whitney U test). In comparison, ulcerative colitis patients had significantly greater metabolism in the descending colon ($p < 0.01$), but values in the ascending colon were not significantly different.

Submucosal specimens metabolised comparatively little butyrate (5.6 (3.2) nmol/μg protein/h) (mean (SD), $n=5$) and glutamine (0.2 (0.2), $n=5$) compared with mucosal specimens (butyrate 66.2 (13.2), $n=5$, $p < 0.01$, glutamine 4.2 (0.6), $n=5$, $p < 0.01$).

The addition of peripheral blood lymphocytes had no significant effect on butyrate metabolism (biopsy specimen with lymphocytes 49.2 (12.3) nmol/μg protein/h, without lymphocytes 52.2 (10.2)) (mean (SD) of five experiments) (95% CI = -22.4 to 24.6), or glutamine metabolism (biopsy specimen with lymphocytes 5.5 (2.2), without lymphocytes 4.5 (1.6)) (95% CI = -2.6 to 3.2).

The addition of 100 μl faecal fluid to a biopsy specimen in culture did not significantly affect metabolism of butyrate (biopsy specimen + faecal fluid 49.7 (10.1) nmol/μg protein/h, biopsy specimen + Krebs-Henseleit 52.2 (5.8)) (mean (SD), five experiments per group) (95% CI = -18.1 to 22.5) or glutamine (biopsy specimen + faecal fluid 5.7 (1.1), biopsy specimen + Krebs-Henseleit 6.8 (2.2)) (95% CI = -3.8 to 2.2). The addition of gentamicin and nystatin to the Krebs-Henseleit buffer had no significant effect on metabolism of butyrate by colonic biopsy specimens (with antibiotics 56 (10.3), without antibiotics 53.4 (7.9)) (95% CI = -16.2 to 21.2).

Incubation with sodium [^{14}C] butyrate in the absence of mucosal biopsy specimens led to an increase in scintillation in the Eppendorf vial of 512 (35) (mean (SD), $n=6$) disintegrations per minute (DPM) above background, which may have been because of the volatility of the butyrate. This was small (<3%) in comparison with the radioactivity produced by substrate metabolism in the presence of mucosal biopsy specimens (range 24 000–115 000 DPM). Incubation with ^{14}C glutamine in the absence of mucosal biopsy specimens gave results that were less than 5% of the values obtained when a biopsy specimen was present.

COLONIC MUCOSAL METABOLISM (Table I)

Controls

No significant difference was found in the rate of metabolism of butyrate by biopsy specimens taken from the ascending and descending colon (Fig 4). Glutamine metabolism was significantly greater in the ascending than in the descending colon ($p < 0.05$, Mann-Whitney U test) (Fig 5). Ratios of butyrate/glutamine metabolism were significantly lower ($p < 0.05$) in the ascending than in the descending colon (Fig 6).

Ulcerative colitis

Butyrate metabolism was similar in ascending and descending colon, and these values were not significantly different from those found in controls (Fig 4). In ulcerative colitis glutamine metabolism was significantly greater in the descending colon than in controls ($p < 0.01$) and there was no significant difference between glutamine metabolism in the proximal and distal colon.

Figure 6: Butyrate/glutamine ratio (colon). In controls, the ratio of butyrate/glutamine metabolism was significantly higher in the descending than in the ascending colon ($p < 0.05$). In ulcerative colitis patients, this relation was lost, and there was no significant difference between controls and ulcerative colitis patients in either site.

The mean ratio of butyrate/glutamine metabolism in the descending colon was greater in controls than in UC, but this did not quite reach significance ($p = 0.09$). Ratios for the ascending colon were similar.

ILEAL MUCOSAL METABOLISM (Table II)

In controls the rate of butyrate metabolism in the ileum was significantly higher than in the ascending colon ($p < 0.05$), and although the mean rate of glutamine metabolism was also higher in the ileum this did not quite achieve statistical significance ($p = 0.06$). The ratios of butyrate/glutamine metabolism were similar in the two regions. In UC the mean rate of butyrate metabolism was greater in the ileum than in the ascending colon, although the difference between the rates was not significant ($p = 0.09$); glutamine metabolism was significantly greater in the ileum than in the ascending colon in UC ($p < 0.05$). No significant difference was found between the rates of metabolism of butyrate or of glutamine by ileal biopsy specimens taken from UC patients compared with controls, and the ratios of butyrate/glutamine metabolism did not differ between UC patients and controls (Figs 7, 8, and 9).

Each data point in Figs 4 to 9 represent the mean of two experiments.

Discussion

In this study mucosal metabolism of butyrate and glutamine in the terminal ileum, ascending colon, and descending colon have been measured in controls and patients with ulcerative colitis. In controls, we found similar rates of butyrate metabolism in the ascending and descending colon, but greater rates of glutamine metabolism in the proximal colon. Thus, although the ratio of butyrate:glutamine metabolism was greater in the normal distal colon this resulted from

TABLE II Metabolism of glutamine and butyrate by ileal mucosa

	Controls (n=8)	Ulcerative colitis (n=6)
Butyrate	111 (57)	135 (56)
Glutamine	15.5 (15.6)	24.1 (16.2)
Butyrate/glutamine	13.6 (11.2)	11.5 (11.1)

Mean figures (SD) for metabolism (nmol/ μ g protein/h) of each nutrient are given.

Figure 7: Butyrate metabolism (ileum). Butyrate metabolism in ileal biopsy specimens taken at colonoscopy (●) or through an ileostomy (■) was similar in controls and ulcerative colitis patients.

Figure 8: Glutamine metabolism (ileum). Ileal glutamine metabolism did not differ significantly between controls and ulcerative colitis patients.

Figure 9: Butyrate/glutamine ratio (ileum). The ratio of butyrate/glutamine metabolism in the ileum of controls and ulcerative colitis patients was similar.

reduced glutamine metabolism rather than increased butyrate metabolism. Rates of butyrate metabolism in controls and ulcerative colitis patients were similar for all three regions studied, but glutamine metabolism was significantly greater in the descending colon in ulcerative colitis. This resulted in a ratio of butyrate/glutamine metabolism that was slightly (but not significantly, $p=0.09$) lower in ulcerative colitis compared with controls.

It has been established that glutamine is an important food for many rapidly dividing cells,¹³ and the increased rate of glutamine utilisation in UC probably relates to the increased rate of proliferation seen in this condition. The higher rate of metabolism of glutamine in the ascending than in the descending colon in controls is in agreement with the findings of Roediger¹⁴ and indeed close examination of his work shows that the difference in the rates of butyrate metabolism in ascending and descending colon was in fact small compared with the difference between rates of glucose metabolism and oxygen consumption. Fleming *et al*¹⁵ have reported no difference in butyrate metabolism between caecal and colonic cells isolated from rats.

It is interesting that butyrate metabolism in controls was greater in the terminal ileum than in the ascending colon and this may suggest that the mucosa here is exposed to the products of bacterial fermentation.

The similar metabolism of butyrate in controls and in patients with ulcerative colitis, is in contrast with previous studies,^{6,7} where butyrate oxidation was impaired in mucosal cells isolated from quiescent and active ulcerative colitis. This discrepancy probably results from the different techniques used to measure metabolism. We have used a technique, whereby mucosal metabolism can be measured in mucosal biopsy specimens, and therefore patients in remission can easily be studied. In addition, our technique avoids potential damage to cells during the cell isolation procedure by EDTA and dithiothreitol. It also seems probable that cells from inflamed epithelium (colitis) will be more prone to toxicity from cytokines than will cells from control tissue and this may have introduced artefactual abnormalities in dispersed colitic cells. The fact that patients with active colitis were more affected than those with quiescent disease in Roediger's studies⁶ could reflect this. A further anxiety over the use of dispersed cells for metabolic studies comes from the finding that the metabolism of fatty acids entering intestinal mucosa from the lumen and plasma is different.¹⁶ Exposure to the surrounding solution of the parts of the cell membrane that are normally in apposition might well affect the metabolism within the cell. Two studies^{8,9} have reported defective butyrate metabolism in epithelial biopsy specimens (as opposed to isolated enterocytes) from patients with ulcerative colitis but these have so far been published only in abstract form. Chapman *et al*⁸ used a similar technique to the one described here, and found significantly lower rates of butyrate metabolism, and non-significant reductions in glutamine and glucose metabolism in UC. Their results on butyrate metabolism therefore concur with the findings of

the dispersed cell experiments^{6,7} and are not in agreement with our findings. The lower rates of metabolism of glutamine and glucose, however, found in UC in that study are not in agreement with either our or Roediger's⁶ findings, so the implications of this report remain unclear. One possible reason for the difference between Chapman's and our results is that their experiments were all performed in the presence of 5 mM glutamine (M Chapman, personal communication) whereas our experiments were carried out in the absence of glutamine. The study by Williams *et al*⁹ reported no metabolism of either butyrate or glutamine by biopsy specimens taken from two UC patients, although there was no report on tissue viability. Some of the reported difference in rates of metabolism could relate to the severity of the underlying colitis.⁶

One further possible explanation for the discrepancy between some of our and Roediger's findings could be that the methods of assessing metabolism were different. For example where he used production of ammonia, we have relied on production of CO₂ as the measurement of glutamine metabolism. It is possible that the route of metabolism of substrates, rather than the rate of their metabolism, is different in the diseased state.

There are some potential disadvantages of using biopsy specimens as opposed to epithelial cell preparations for metabolic studies. It could be argued that different specimens could have different proportions of actively metabolising (mucosa) and inactive (lamina propria) tissue. For this reason we used a constant size of biopsy forceps throughout the study and reproducibility of the assays was satisfactory. Any error as a result of variation in the proportion of active and inactive tissue between patients (such as might result if one patient's mucosa was atrophic) should be alleviated by measuring glutamine metabolism in adjacent biopsy specimens and examining the ratio of butyrate/glutamine metabolised. The validation experiments suggested that lymphocytes probably contribute little to overall measured metabolism. Binder¹⁷ found an excess of 1000 cells/mm² lamina propria in mildly active UC compared with controls, and assuming specific gravity=1 and that lamina propria accounts for about half the weight of a biopsy specimen, the excess number of cells in lamina propria of a 10 mg specimen=150 000. Selby *et al*¹⁸ isolated an excess of 44×10^6 mononuclear cells/g UC mucosa, or 440 000 per 10 mg biopsy, so both these estimates are considerably smaller than the number of lymphocytes studied with each biopsy (2.56×10^6).

One further effect worth considering is the bowel preparation before harvesting of the tissue. Ardawi and Newsholme¹⁹ have shown that starvation reduces the rate of metabolism of glutamine, but not that of ketone bodies by colonocytes. By avoiding variations in bowel preparation (apart from the two patients with ileostomies) any such effect due to a change in diet should be equivalent in the UC and control patients.

It was notable that butyrate metabolism was

greatest at a concentration of 1 mM with a fall off in metabolism at higher concentrations. Concentrations of 10–15 mM are present in the colonic lumen,^{20–22} but separated cells and biopsy specimens might well react differently from an intact mucosal surface. Morita *et al*²³ have reported toxic effects of sodium butyrate above 2 mM in a colon cancer cell line and it may be that previous studies have used concentrations of butyrate that are higher than optimal.

Studies have suggested that both diversion colitis²⁴ and ulcerative colitis²⁴ respond to rectally given short chain fatty acids, and recently a controlled trial of butyrate enemas in ulcerative colitis showed significant improvement in clinical and pathological parameters over placebo.²⁵ Vernia and colleagues²⁶ have shown reduced concentrations of butyrate in the faecal water of patients with ulcerative colitis, but not in Crohn's colitis. A reduced short chain fatty acid concentration has also been reported in pouch contents from patients with pouchitis compared with those without.²⁷ The response of ulcerative colitis to butyrate administration suggests that the mucosa is able to utilise butyrate adequately, and makes it more plausible that the colitis results from a deficiency of, rather than an inability to metabolise, butyrate.

These studies show that mucosal metabolism can be assessed effectively using biopsy specimens without the need for epithelial cell purification. The increased metabolism of glutamine by the distal colon in ulcerative colitis seems probably to reflect a response to inflammation and consequent hyperplasia and is in keeping with evidence that respiratory chain enzymes are increased in ulcerative colitis.²⁸ It seems unlikely from this work that ulcerative colitis is a result of a primary defect in butyrate metabolism.

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Consequently, growing older, educational standard, and social habits could participate in atrophic gastritis but not in the same way as for the acquisition of *H pylori*.

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BOOK REVIEW

Diseases of the Gut and Pancreas. Edited by J J Misiewicz, R E Pounder, C W Venables. 2nd ed. (Pp 1160; illustrated; £99.50.) Oxford: Blackwell Scientific, 1994.

The evolving relationship between a gastroenterologist who has acquired a new textbook can be compared with a car owner and his new car. Both objects look bright and new at the beginning. It is a different and interesting experience to 'get into them' but, quite soon, the novelty wears off. Occasionally, when they are particularly needed, either can let you down.

With textbooks, it is perhaps a little unfair to come to a hurried judgment on a first edition. Indeed, there may be a case for sparing detailed critical attention until the second edition has come along. If the textbook is not up to scratch, it will not actually reach a second edition but, by the time a revised version does hit the market, authors and editors will have had an opportunity to refine the product.

I had a copy of the first edition of this book and, judging by its rather osteoporotic spine, I seem to have used it quite a lot. It was certainly unique on my bookshelf in being the only substantial textbook of gastroenterology that could be easily held and read in the hand. Its length and breadth were barely larger than the original size of this journal (deceased 1989). The second edition has grown in length and breadth (about the dimensions of the modern *Gut* - 1990 onwards) and has slightly contracted in width. How good is this new model?

It is very much a textbook of clinical gastroenterology, as its title would imply. Furthermore, it is emphatically in a single volume, and I have to say it is my strong personal view that it is very much a frustration of modern life to have to read multi-author chapters in multi-editor texts that come in multiple volumes. The growth of knowledge is inexorable, but it is good to see this matched by editorial excellence, which makes the book both readable and undaunting. For example, the whole of inflammatory bowel disease is covered in 90 pages with ulcerative

and Crohn's disease, each having three chapters on their clinical features, medical management, and surgery respectively. I have not seen the clinical aspects of inflammatory bowel disease covered so well given this sort of space constraint, but there is necessarily some sacrifice of detail, particularly the pathogenesis (especially immunology) of these conditions. One accepts that a single volume will inevitably concentrate on the most clinically relevant areas but, since its first edition, there has been a really substantial expansion in the number of references for each chapter with almost all increasing by at least half and, in many cases, doubling.

The publisher has served the authors and editors really well. The various hierarchies of headings and subheadings are very easy on the eye. The figures and tables are beautifully produced and the x rays are compellingly clear. As usual, black and white histology is disappointing and it seems curious that there are so many photographs of extremely thin, naked people. Sadly, gastrointestinal disease may cause patients to become thin and I am not sure how much is gained from reminding us of this so visually.

Inevitably, any reviewer will find 'highs' and 'lows'. I have already mentioned my liking of the section on inflammatory bowel disease, but I also enjoyed the chapter on nutrition. The section entitled 'Colonic Polyps' was a little disappointing. This is surely an area of keen interest for gastroenterologists. The table of polyposis syndrome made no mention of hereditary non-polyposis colon cancer or Lynch syndrome. Perhaps a detailed discussion of molecular genetics was outside the scope of the book, but I think this most exciting area of gastrointestinal research could have merited a few lines. By contrast, the immunogenetics of coeliac disease is discussed but, in this otherwise very readable chapter, it is surprising to find no discussion of the widening spectrum of the condition.

This is an excellent book. It is written by practising clinicians for practising clinicians and, as such, is not surpassed.

IAN FORGACS

NOTES

Sir Francis Avery Jones BSG Research Award 1995

Applications are invited by the Education Committee of the British Society of Gastroenterology who will recommend to Council the recipient of the 1995 Award. Applications (**fifteen copies**) should include:

(1) A manuscript (2 A4 pages *only*) describing the work conducted.

(2) A bibliography of relevant personal publications.

(3) An outline of the proposed content of the lecture, including title.

(4) A written statement confirming that all or a substantial part of the work has been personally conducted in the United Kingdom or Eire.

Entrants must be 40 years or less on 31 December 1995 but need not be a member of the BSG. The recipient will be required to

deliver a 40 minute lecture at the Spring meeting of the Society in 1995. Applications (**fifteen copies**) should be made to: The Honorary Secretary, BSG, 3 St Andrews Place, London NW1 4LB by 1 December 1994.

Gastroenterology and liver disease

The 20th anniversary of Annual Topics in Gastroenterology and Liver Disease will be held on 5-7 October 1994 in Baltimore, Maryland, USA. Further information from: Program Coordinator, Johns Hopkins Medical Institutions, Office of Continuing Education, Turner Building, 720 Rutland Avenue, Baltimore, Maryland 21205, USA. Tel: 410 955-2959.

Gastrointestinal motility

The Eighth Biennial Meeting of the American Motility Society will be held on 20-23 October 1994 at Wintergreen Resort, Wintergreen, Virginia, USA. For further information contact the Office of Continuing Medical Education, Box 368, University of Virginia Health Sciences Center, Charlottesville, Virginia 22908, USA. Tel: 804 924 5310.

Liver diseases

The American Association for the Study of Liver Diseases will hold a series of meetings during November 1994 in Chicago, USA. Further information on these meetings is available from: AASLD, Registration Manager, 6900 Grove Road, Thorofare, NJ 08086, USA. Tel: 609 848 1000, extn 213.

Pancreatic diseases

The Combined Meeting of the International Association of Pancreatology and American Pancreatic Association will be held on 2-4 November 1994 in Chicago, USA. Further information from: Linda Bakken, GI Unit 2-424, Mayo Clinic, 200 1st Street SW, Rochester, MN 55905, USA. Tel: 507 255 4303; fax: 507 255 6318.

Therapeutic endoscopy

The Chinese University of Hong Kong and the Hong Kong Society of Digestive Endoscopy will hold the Ninth International Workshop on Therapeutic Endoscopy on 6-8 December 1994 in Hong Kong. Further information from: Dr Sydney Chung, Endoscopy Centre, The Chinese University of Hong Kong, Prince of Wales Hospital, Shatin, NT, Hong Kong. Tel: 852 636 2233; fax: 852 635 0075.

Correction

An authors' error occurred in the paper by Dr I A Finnie *et al* (*Gut* 1993; 34: 1552-8). The units of metabolism for butyrate and for glutamine throughout the paper should have been nmol substrate metabolised/h/mg protein and not, nmol/h/ μ g protein as stated. The ratios for butyrate/glutamine metabolism, the statistical analysis, and the conclusions remain unchanged.