LETTERS TO THE EDITOR

Colonic epithelial metabolism in ulcerative colitis

EDITOR,—The paper by Drs Finnie, Taylor, and Rhodes (published in this issue of *Gut*) is an important contribution to the study of colonic mucosal metabolism, now evaluated by colonic biopsy specimens containing both epithelial cells and lamina immune cells, rather than isolated colonic epithelial cells devoid of immune cells. Unfortunately the current method and isolated cell system previously reported¹ have not been compared with regard to metabolic performance.

The results of the biopsy technique are somewhat at variance with findings that we and others²⁻⁴ have made. The study by Finnie *et al* shows: (a) concentration of 5 mmol/l of butyrate to have an adverse effect on mucosal tissue; (b) no changes of butyrate oxidation have been found in ulcerative colitis compared with controls; and (c) no significant glutamine utilisation was found in lymphocytes that are known to utilise glutamine.⁵ These are important metabolic differences, which are puzzling but for which some explanation comes to mind. Butyrate metabolism in biopsy specimens may have been (a) suboptimal as dithiothreitol, which universally increases oxygen consumption¹ was not present in the incubation medium; (b) the degree of radioactivity was considerably 'hotter' with biopsy specimens than isolated cells: radioactive swamping comparative with weight of epithelial cells may therefore have produced an unsatisfactory precursor product relation⁶; (c) despite current findings on circulating lymphocytes, lamina immune cells particularly those in ulcerative colitis would be metabolically more activated in ulcerative colitis cases than control cases⁷ and these may have affected patterns of substrate metabolism. The great advantage of isolated epithelial cells is that they contain almost no immune cells, which usually will display some metabolic activity in the biopsy method.

Therapeutically butyrate has been beneficially used in humans at a concentration of 100 mmol/l in four separate studies and no adverse effects reported on colonocytes. We have again checked whether 10 mmol/l impairs butyrate oxidation in isolated human colonocytes and cannot find an effect as now reported by Finnie *et al*. Further metabolic studies in this field are needed in which the above points should be considered. The biopsy techniques and isolated colonic epithelial cells prepared from the same colonic segment deserve comparison before an alternative conclusion can be drawn about butyrate metabolism in ulcerative colitis.

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Reply

EDITOR,—We are grateful for Dr Roediger's comment. It would certainly be useful to be able to compare our cultured biopsy specimen technique with his isolated cell technique in the same patients. This would however be difficult in patients with quiescent colitis in whom resection specimens would not be available. We have attempted metabolic studies in epithelial cells extracted from colonoscopic biopsy specimens but found that reproducibility was poor and abandoned this approach. We were particularly keen to develop a technique that could be applied to patients in remission (and also to ileal mucosa) to avoid the usual difficulty of trying to assess whether any changes found are merely secondary to inflammation rather than of primary importance. We were also anxious that the process of isolating epithelial cells might result in greater damage to cells obtained from diseased epithelium than from controls.

Our technique shows good reproducibility and confirms the high rate of butyrate metabolism by the normal colonic mucosa as shown by others. It also shows increased metabolism of glutamine by the distal colonic mucosa in ulcerative colitis, which resulted in a lower ratio of butyrate: glutamine metabolism. It is possible that some of the apparent discrepancies may have resulted from the fact that other studies of butyrate were carried out in the presence of glutamine¹ or dithiothreitol and EDTA.² Further studies would certainly be useful to assess these methodological differences.

We feel it is very unlikely that the increased metabolism of glutamine in ulcerative colitis is the result of metabolism by inflammatory cells. It is not correct to say that our technique failed to show any metabolism of glutamine by lymphocytes. When lymphocytes were added to mucosal samples in numbers roughly equivalent to those likely to be present in excess in inflamed mucosa there was an increase in metabolism from (mean (SD)) 4.5 (1.6) nmol/ μ g protein/h to 5.5 (2.2) but this was not statistically significant. In parallel studies not included in the paper five aliquots each of 1×10^6 lymphocytes studied alone showed glutamine metabolism equivalent to 1.2 (1.1) (mean (SD)) nmol/h using the same method. The number of lymphocytes in a biopsy specimen is likely to be far outweighed by the number of epithelial cells, however,³ and the metabolism of glutamine is greater in the second.⁴ Although Ardawi and Newsholme suggested that glutamine metabolism by lymphocytes could increase during proliferation⁵ they suggested that the same could also apply to colonocytes.⁴

Although the amount of radioactivity used in our study (1 μ Ci for 10 mg tissue) was about eight times greater per unit weight of tissue than Roediger used (0.25 μ Ci for 5 mg tissue = 20 mg dry weight), up to 5% of the [¹⁴C] butyrate was retrieved as ¹⁴CO₂, and this is a reasonable indication that 'swamping' did not occur.

The response of ulcerative colitis to rectal butyrate suggests that the epithelium is able to

use this effectively as a substrate in ulcerative colitis, and whether 100 ml of a 100 mM butyrate enema exposes epithelial cells to a concentration that is closer to the one we used (1 mM) in tissue culture or that used by Roediger (10 mM) in cell culture is not known. At acid pH 850 mM sodium butyrate has been shown to be considerably colitogenic in mice.⁶

We agree that the isolated cell model has potential advantages over our system, but suspect that these advantages may be outweighed by the potential problem of inflicting damage upon cells during their isolation. Further studies comparing the two methods are warranted; but these should ideally be carried out in quiescent ulcerative colitis in which case a technique for studying cells isolated from biopsy specimens will need to be evaluated.

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Helicobacter pylori positive resistant duodenal ulcers

EDITOR,—Professor Bianchi Porro *et al* must be congratulated upon their elegant study on the use of colloidal bismuth subcitrate (CBS) plus antibiotics in *Helicobacter pylori* positive resistant duodenal ulcers (*Gut* 1993; 34: 466-9). Their article, however, while providing new insights into an unsolved problem elicits a number of further questions.

In my opinion it would have been preferable to have included a group of patients with resistant ulcers treated with CBS alone. Indeed the effectiveness of the drug in promoting healing of refractory duodenal ulcers is not unanimously shown.¹ On the other hand, the possibility that CBS may, by itself, be superior to sucralfate in this particular type of ulcer cannot be dismissed, even though the comparative trial between the two drugs in non-resistant duodenal ulcers quoted in Professor Bianchi Porro's paper is unreliable because of the great numeric imbalance between treatment groups.²

Moreover, if antibiotics, as the authors seem to suggest, exert a direct ulcer healing activity, which is independent of their effect on *H pylori*, then either a group with CBS without antibiotics should have been included or antibiotics should have also been added in the sucralfate group. A treatment regimen of sucralfate plus antibiotics has actually been reported to be highly effective in resistant duodenal ulcers.³

The poor treatment response to sucralfate (47%) obtained in Bianchi Porro's study is in apparent disagreement with our earlier, uncontrolled report of an 81% success rate with that anti-ulcer agent in refractory duodenal ulcers.⁴ Of course, at that time, virtually nothing was

known of the possible role of *H. pylori* in duodenal ulcer disease and the *H. pylori* state of those patients cannot be determined retrospectively.

It is possible that most subjects in our series were *H. pylori* negative, which would explain the higher healing rates compared with Bianchi Porro's experience. It is more probable that our patients were either a mixture of *H. pylori* positive and negative with ulcers or mostly *H. pylori* positive, which does not help to explain the reasons for this discrepancy.

By far the most intriguing question raised by Bianchi Porro's study is whether resistance to standard anti-ulcer treatment can be related to the presence of *H. pylori*. In Wagner's study with bismuth subsalicylate, 14% of duodenal ulcers did not heal despite *H. pylori* clearance and 65% of healed ulcers had persistent *H. pylori* infection,³ suggesting that this might not be the case.

Eradication by means of a more complete treatment regimen rather than mere clearance of the micro-organism may have a bearing on the subsequent relapse rate but can hardly account for the superior effects in the short term. A role for *H. pylori* in some cases of refractory duodenal ulcers remains, however, an attractive hypothesis to which Professor Bianchi Porro's data certainly give support.

At the present time omeprazole is the anti-ulcer drug that provides the most striking results in the treatment of resistant duodenal ulcers.⁶ Its efficacy has generally been related to sustained and powerful acid inhibition, but the drug is also known to exert a clearing effect on *H. pylori*, if not to eradicate the micro-organism.⁷ Further studies are needed to discriminate between the roles of acid suppression and *H. pylori* inhibition in the successful use of omeprazole for refractory duodenal ulcers.

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EDITOR.—Professor Bianchi Porro *et al* are to be congratulated on their interesting paper (*Gut* 1993; 34: 466-9). It is not surprising that only six of 15 refractory duodenal ulcers were healed after four weeks' treatment with sucralfate 4 g/day. Non-refractory ulcers require six weeks' or up to 12 weeks' treatment for healing. It is interesting that, in the two patients with unhealed ulcers after four weeks' treatment with bismuth subcitrate plus

amoxycillin and tinidazole, both healed with a further four weeks' treatment with sucralfate. It is known that sucralfate has no direct action on *Helicobacter pylori*. *H. pylori*, however, cannot exist in the duodenal mucosa in the absence of gastric metaplasia.

In the small study we reported in 1989^{1,2} duodenal gastric metaplasia completely disappeared or became minimal in eight of 11 (73%) patients with healed duodenal ulcers after one year's maintenance on sucralfate 1 g twice daily. This compared with only five of 14 (34%) of patients who had been on one year's maintenance with cimetidine. In the subsequent two years, two of 11 in the sucralfate group relapsed, compared with nine of 13 in the cimetidine group. In the absence of gastric metaplasia, no *H. pylori* organisms were seen by light or electron microscopy in the duodenal mucosa and they were only very rarely seen when there was minimal gastric metaplasia.^{3,4}

These findings would suggest that longterm maintenance treatment with sucralfate, by enhancing mucosal resistance to *H. pylori*, may be an alternative means of eliminating the organism and reducing the relapse rate.⁵

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EDITOR.—Professor Bianco Porro *et al* (*Gut* 1993; 34: 466-9) have shown that eradication of *Helicobacter pylori* in patients with duodenal ulcer disease resistant to H₂ blockers provides effective healing with low relapse rates at one year. They also state that single drug treatment with colloidal bismuth subcitrate or omeprazole heals at least 80% of these resistant ulcers within four weeks.

We describe a 30 year old man with an 11 year history of epigastric pain unresponsive to drug treatment. He smoked 20 cigarettes daily, and there was no history of ingestion of non-steroidal anti-inflammatory drugs or excessive alcohol intake. The three gastroscopies performed during this period reported either erosive duodenitis or 'salt and pepper' duodenitis. Duodenal brushings did not show any pathogens. Treatment regimens included colloidal bismuth citrate 120 mg four times daily, cimetidine 400 mg twice daily with sucralfate 1 gm four times daily, ranitidine 150 mg twice daily, pirenzepine 50 mg three times daily and omeprazole 60 mg once daily, but none pro-

duced symptomatic relief. A further gastroscopy again reported 'salt and pepper' duodenitis while quadrantic antral biopsy specimens showed chronic inflammation and *H. pylori* like organisms. He was then treated with colloidal bismuth citrate 120 mg four times daily for two months with amoxycillin 500 mg four times daily and metronidazole 400 mg three times daily for the initial two weeks. He had an excellent symptomatic response and remains asymptomatic one year after completing the course of treatment.

H. pylori is probably a significant factor in the cause of erosive duodenitis in this patient. Symptomatic erosive duodenitis seems less responsive than duodenal ulcers to gastric acid inhibition.^{1,2} Persistent duodenitis may also be a marker for early relapse of duodenal ulceration.³ Eradication regimens for *H. pylori* should be considered at an early stage of treatment of symptomatic erosive duodenitis.

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Reply

EDITOR.—Thank you for giving us the opportunity of replying to the comments on our article.

Dr Guslandi raises two important points in his letter: firstly, the lack of a group of refractory duodenal ulcers treated with CBS alone and secondly, the possibility of reasons other than anti-*H. pylori* activity, for the superiority of triple therapy over sucralfate alone. We share his concern about the possibility that CBS alone may be superior to sucralfate in these patients and that it would have been interesting to include such a treatment group in the trial. Unfortunately, comparative trials between the two drugs in refractory duodenal ulcer patients are lacking, and only one controlled study comparing CBS with sucralfate in non-resistant patients exists to date.¹

As far as the reasons for the high efficacy of the triple therapy in refractory duodenal ulcers are concerned, we feel that this is mainly because of its anti-*H. pylori* activity. Indeed, the rate of healing was significantly higher in those patients where *H. pylori* was eradicated after treatment than in those who had a persistent infection. This view is also supported by two recent trials in non-resistant duodenal ulcers, which report that adding antibiotics to an anti-ulcer regimen accelerates the healing of ulcers.^{2,3} Therefore, in our opinion, an eradication regimen should be considered as the treatment of choice in the presence of an *H. pylori* positive refractory duodenal ulcer, as it is capable not only of healing the anatomical lesion but also of changing its natural history. Tovey *et al* place great emphasis upon the shortness of initial acute treatment (four weeks) to explain the low healing activity of sucralfate alone, but we feel that in most of the existing trials on refractory duodenal ulcers the length of short term treatment was predetermined at four weeks as it is clear that prolonging the treatment (whatever drug is