We agree that not all patients with duodenal ulcer disease secrete more acid than normal. Patients with duodenal ulceration, however, on average produce more acid than normal.^{1,2} As gastric metaplasia is an almost constant finding at the margins of duodenal ulcers, increased acid flux may at least be one contributory factor to the development of gastric metaplasia. This is substantiated by many experimental findings and clinical studies.³⁻⁶ Indeed, the high prevalence of gastric metaplasia in dyspeptic patients in general suggests that other, still unknown factors, may also be involved.

The short term lack of regression of gastric metaplasia after eradication of *H pylori* may also point to the multifactorial nature of gastric metaplasia, including ongoing robust acid secretion during the first year after eradication of *H pylori*.⁷⁻⁹ Evidence emerges that gastrin mediated acid output regresses one year after eradication of *H pylori* in duodenal ulcer disease.¹⁰ Up to now, we cannot exclude that gastric metaplasia may be slowly reversible in the long term as has been seen after vagotomy.¹¹ Again, other factors than acid and *H pylori* infection may be responsible for the persistence of gastric metaplasia in other patients.

We do not agree with Savarino *et al* that the pathogenic relevance of gastric metaplasia for duodenal ulcer patients is diminished by the fact that gastric metaplasia failed to regress in the short term follow up after eradication of *H pylori*. In our opinion, the almost invariable presence of gastric metaplasia at the margins of duodenal ulcers shows that both gastric metaplasia and (ulcerogenic strains of) *H pylori* are required for the development of duodenal ulceration. Longer follow up studies will be necessary to find out if, to what extent, and with what velocity gastric metaplasia regresses in duodenal ulcer and non-duodenal ulcer subjects.

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Risk factors for the development of gall bladder disease

EDITOR,—I enjoyed reading the article by Murray *et al* (*Gut* 1994; 35: 107-11). I am surprised that the authors have published data where there is no confirmatory evidence of gall stones in most of their patients. I agree it is unlikely that the general practitioners in the study changed their diagnoses in patients with upper abdominal pain on the basis of smoking habit. On the other hand it is quite conceivable that smokers experienced more episodes of acute upper abdominal pain because of peptic ulceration or reflux disease.

With 80% of patients not even admitted to hospital and no indication of the percentage of patients who had confirmed gall stone disease this paper cannot fully consider the effect of smoking on gall stone disease. It tells us that smokers experience more upper abdominal pain than non-smokers. Whether or not this results from gall stones is another question, which remains unanswered.

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Ileal and colonic epithelial metabolism in quiescent ulcerative colitis

EDITOR,—The paper by Drs Finnie *et al* (*Gut* 1993; 34: 1552-8) showing that mucosal biopsy specimens from sufferers of quiescent ulcerative colitis possess a normal ability to oxidise butyrate is in direct contradiction to our findings where we showed a reduced capability.¹ We used an almost identical technique, which has been validated and exhibits a similar degree of reproducibility.^{2,3} We would concur with the conclusions of Finnie *et al* on tissue viability, effects of lymphocytes, and of contaminating bacteria (although assaying 100 µl of faecal fluid would not ascertain whether there were different adherent bacteria on the mucosal surface in disease and controls) on the assay. It is difficult to account for the differences in the results but we would like to make a few points to complement those of Professor Roediger (*Gut* 1993; 34: 1646).

The rates of oxidation of glutamine and butyrate found by Finnie *et al* were about a 100-fold greater than ours using biopsy specimens, and other workers using isolated colonocytes.³ Working through the calculations of Finnie *et al* it would seem that the protein content of their specimens was far less than would be expected from the wet weights of the specimens.

This problem may be illustrated by

reference to the data shown in Fig 2 where specimens of about 10 mg wet weight were incubated in 1 ml of medium containing 1 mM [¹⁻¹⁴C] labelled butyrate (1 µmol butyrate in total) for up to three hours. After two hours about 200 nmol of carbon dioxide were produced for each µg of tissue protein present in the incubation. As the complete oxidation of butyrate results in four molecules of carbon dioxide this represents the utilisation of 50 nmol of butyrate per µg tissue protein. At this rate of utilisation all the butyrate in the incubation medium would have been oxidised in two hours by a specimen containing 20 µg of protein. It is difficult to envisage that a 10 mg specimen could yield no more than 20 of µg protein.

This same problem can be considered from another angle, without assuming complete oxidation of the butyrate. One µCi (37 KBq) of labelled butyrate was added to 1 µmol of butyrate, thus the specific activity was 2220 disintegrations per minute (DPM) per nmol. Finnie *et al* quote a range of 24 000-115 000 DPM for butyrate. This represents 11-52 nmol/10 mg wet weight/2 hours and converts to 0.55-2.6 nmol/mg wet weight/hour. From Table 1 and Fig 4 the mean rate of butyrate oxidation is about 75 nmol/µg protein/hour. Thus a specimen weighing 10 mg wet weight would be expected to yield 0.3-0.07 µg of protein. In our experience a colonic biopsy specimen with a wet weight of about 10 mg is equivalent to about 800 µg protein weight, which is much closer to the ratio found by other workers.⁴

It is difficult to comment in detail without seeing the raw data but it is possible that the division of the biopsy sample into very small pieces led to the shedding of a significant amount of material as cell clusters during the incubation period. The use of perchloric acid to terminate the reaction and liberate the carbon dioxide may then also have resulted in the precipitation of protein, which was lost to the assay.

All our experiments, as Finnie *et al* correctly pointed out, were performed in the presence of 5 mM glutamine and 5 mM butyrate. It is known that the presence of 5 mM butyrate suppresses the rate of oxidation of glutamine in the rat colonocyte.⁵ If this holds true for the human it is difficult to draw conclusions from the work of Finnie *et al*, as in vivo both glutamine and butyrate will be present.

While we agree that there is evidence to suggest that butyrate concentrations greater than 2 mM may be toxic to certain cell lines there is no evidence to suggest that concentrations of less than 40 mM are toxic to isolated colonocytes.⁶ Finnie *et al* found that using concentrations of either 1 mM or 5 mM butyrate had no significant effect on the ability of the specimens to oxidise butyrate (Fig 3). In our experiments we used a butyrate concentration of 5 mM.³

The finding that the ratio of butyrate/glutamine oxidation in the distal colon is greater than in the proximal colon in the control group and that this relation is lost in the diseased group suggests that this second group has a lower dependency on butyrate and is more reliant on glutamine as a fuel source. It may be speculated that this is a response to an inability to oxidise butyrate and explains why inflammation is more commonly seen in the distal rather than proximal colitic colon.

The results from the ileal mucosal biopsy specimens are at variance with our previously published results⁷ and we wonder if con-

clusions can be drawn from specimens taken from only six patients of whom two had ileostomies. The results show a coefficient of variation of about 50% and the risks of a type 2 statistical error must be very high.

In conclusion it is important that Finnie *et al* suggest an explanation as to why they found such a low protein weight to wet weight ratio. Until they do so it is very difficult to interpret their data.

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Reply

EDITOR,—We thank Mr Chapman for his comments regarding our paper and are grateful to the editor for the opportunity to respond to the points raised.

In response to his first point, he has correctly identified an error on our part. The units quoted throughout our paper for butyrate and glutamine metabolism should have been nmol substrate metabolised/h/mg protein. We apologise for the confusion that has resulted. The statistical analysis and conclusions for all the data remain as stated.

The critical difference between our study and that of Chapman *et al*¹ is that we have examined butyrate metabolism in the presence of glucose but not glutamine, whereas their study was performed in the presence of all three energy substrates. Our own studies and those of Roediger² (but not those of Chapman *et al*) have shown increased metabolism of glutamine in ulcerative colitis. It seems likely that this may be a reflection of hyperplasia, as rapidly dividing cells have increased metabolism of glutamine.³ In the absence of glutamine we have failed to find any defect in metabolism of butyrate, and suspect that the results of Chapman *et al* may reflect the predilection of the mucosa for glutamine at least as much as the reduced ability to metabolise butyrate. To some extent a similar conclusion can be reached from part of Roediger's original study. He showed that the rate of oxygen uptake in the presence of butyrate as the sole energy substrate was normal in quiescent ulcerative colitis and only reduced in acute colitis, and although production of carbon dioxide from butyrate was reduced in ulcerative colitis, the most

striking change in the contribution of different fuels to total oxygen consumption was the increased metabolism of glucose in quiescent colitis.

With regard to the appropriate choice of glutamine concentration there is no clear answer. As Chapman *et al* themselves point out there is likely to be little or no glutamine available from the colonic lumen because it is so readily utilised by colonic flora,¹ and peripheral blood concentrations of glutamine are less than 0.5 mM⁴, so culture in the presence of 5 mM glutamine cannot be considered physiological.

We were interested by the results from ileal mucosal biopsy specimens reported by Chapman *et al* in their abstract.⁵ We would suspect that the reduced metabolism of butyrate that they have shown in the terminal ileum of patients undergoing colectomy, presumably for severe colitis, might be to some extent a reflection of the severe illness combined with backwash ileitis. For this reason we chose to study ileal biopsy specimens obtained at colonoscopy from patients in clinical remission. The figures for coefficients of variation we published were for all specimens, ileal and colonic included (20% for butyrate metabolism and 23% for glutamine metabolism). The coefficients of variation for glutamine metabolism in ileal biopsy specimens was 20% and for butyrate 18%, not 50% as stated by Chapman *et al*. There was, if anything, a very slight trend towards increased butyrate metabolism in ulcerative colitis in our study. We have subsequently increased the numbers of ileal biopsy specimens studied to 18 in all and the conclusion remains the same.

There clearly are some interesting differences in mucosal metabolism in ulcerative colitis but our view is that most of these differences are likely to reflect changes occurring secondary to hyperplasia.

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Risk factors for *Helicobacter pylori*

EDITOR,—The findings of the EUROGAST Study Group on risk factors for *H pylori* infection (*Gut* 1993; 34: 1672-6) interested us because of the relation between this infection and gastritis. The principal finding of the group was a positive correlation between infection and low educational standard. They suggested this showed that social class was a relevant factor. In 1966 we found¹ a correlation between atrophic gastritis on gastric biopsy and social class in a series of 221 patients suffering from non-ulcer dyspepsia. The prevalence of gastritis increased with descending social class, and increased

with age in all classes, becoming roughly equal in each class at 50 years and over.

Unlike the EUROGAST study our data suggested a positive correlation also between atrophic gastritis and excessive cigarette smoking, excessive alcohol consumption, and drinking hot tea.

The study by Vincent *et al* (*Gut* 1994; 35: 313) showed a high prevalence of *H pylori* in children cared for in a medical centre for mentally retarded children. The authors thought that factors related to close contact with other children were probably the cause of the high prevalence.

We were puzzled by our findings nearly 30 years ago. It is of interest that Bateson² in his review of *H pylori* infection mentions its higher prevalence, and at an earlier age, in the developing world. Perhaps, if there is a relation between atrophic gastritis (and *H pylori* infection) and social factors it is mediated through interacting elements of living conditions probably associated with low grade education: financial hardship, restricted accommodation with people in close proximity and possibly sharing food and cooking utensils, poor hygiene, and low social class.

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Reply

EDITOR,—Based on their findings, Edwards and Coghill suggest that social factors implied in the spread of *H pylori* infection could explain the epidemiological pattern of atrophic gastritis.

Crowding and promiscuity are probably the ultimate risk factors of *H pylori* infection. These conditions are mediated by a low social economic class, which is a good risk marker but not a causal factor of infection. Our study showed that when they have been living in the same conditions for years, cohabiting children from different ethnic and social origins attain the same level of risk for *H pylori* infection. Furthermore, the high seroprevalence known in gastroendoscopists¹ shows that infection can be acquired even in hygienic conditions.

This is not in disagreement with the social distribution of atrophic gastritis. *H pylori*, which is generally acquired in childhood, induces a chronic inflammatory reaction for years, but epithelial changes of atrophy and metaplasia are not constant and could vary with the host and circumstances. Atrophic lesions are uncommon in infected children, and it has been shown in adults that the mucosal colonisation area of *H pylori* can be larger than the area of chronic active gastritis.² Moreover, an aggravation of mucosal lesions of *H pylori* associated gastritis can be found after absorption of non-steroidal anti-inflammatory drugs (NSAIDs).³

Atrophic gastritis found in adults might be a multifactorial condition resulting from *H pylori* infection (as a necessary but not sufficient factor) associated with consumption of gastric toxic agents, such as NSAIDs, alcohol, tobacco, spiced food, etc.