LETTERS TO THE EDITOR

Caffeine phenotyping in patients with familial adenomatous polyposis may shed light on sporadic colon cancer and acetylator status

EDITOR,—The age at which patients with familial adenomatous polyposis (FAP) develop colorectal cancer (CRC) is variable, as is the development of extracolonic tumours in the syndrome. This could be due to the effect of different mutations in the responsible gene,¹ environmental factors or the influence of risk modifier genes. Enzymes that metabolise dietary carcinogens such as *N*-acetyltransferase2 (NAT2) and cytochrome P450 CYP1A2 are potential modifier genes and fast metabolisers for NAT2 and CYP1A2 have been reported to be at greater risk of sporadic CRC.² In addition bile from FAP patients has been found to contain higher concentrations of carcinogens³ than that from controls. Therefore, Spigelman *et al* (*Gut* 1995; 36: 251–4) have examined the frequency of phenotypes for NAT2 and CYP1A2 in FAP against controls and also examined the influence of colectomy on the caffeine metabolite ratios used for phenotyping. We believe that a re-examination of their data questions the use of the phenotyping method for NAT2 in these patients and may help to explain the excess risk for fast acetylators seen in phenotyping studies of colorectal cancer.

NAT2 has a well characterised polymorphism⁴ and patients can be classified as fast or slow acetylators either by means of phenotyping, using a probe drug such as caffeine, or by genotyping using a simple PCR based assay for the four common alleles. In healthy young subjects the two methods are well correlated.⁴ In their paper Spigelman *et al* state that there is no significant difference in the value of the metabolic ratio for NAT2 phenotype in a group of six patients before and after surgery. However, if we re-examine the data from Fig 2 and look at the apparent phenotype in each subject rather than the metabolic ratio it would seem that one subject had changed from a slow to a fast acetylator after colectomy, one has done the reverse, and another who seems very close to the cut off point of 0.48 before surgery is now clearly a fast acetylator. Thus possibly as many as 50% of subjects seem to have changed their phenotype in this small group of subjects. If this finding were repeated in a larger sample it would invalidate the conclusion that there are more slow acetylators in the FAP group.

While fast acetylator status has been associated with greater risk for sporadic CRC in a number of phenotyping studies in white subjects,² two genotyping studies in Japanese subjects found no increased risk.^{5,6} It may be that this is due to different dietary risk factors or the high (90%) prevalence of fast acetylators in Japan but clearly it could also result from a disease effect on the phenotyping procedure causing misclassification of patients in the phenotyping studies in white subjects. We believe that phenotyping may be inaccurate in people with concurrent diseases and that the study by Spigelman *et al* gives some support to this theory. In contrast, genotyping offers a quick and simple

means of assessing acetylator status, which is unaffected by concurrent illness or drugs. Until further studies have validated phenotyping in patients with FAP or CRC we believe that genotyping is the method of choice for NAT2 analysis and a genotyping study is required to establish whether fast acetylator status is truly a risk factor for this disease in white subjects.

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Reply

EDITOR,—We thank Dr Welfare and colleagues for their valued remarks. We entirely agree that genotyping studies are required to evaluate further the role of pharmacogenetic polymorphisms in carcinogenesis, and said as much in our paper. Moreover, the context in which we made this point shows just why we did not use genotyping in our study – the techniques, which are now 'quick and simple', were not available at the time our work was conceived and executed. We have since, however, been able to use genotyping¹ to investigate this question, and have found, for example, that the GST Mu polymorphism (GST Mu being a carcinogen detoxifying enzyme system) is unequally distributed between patients with familial adenomatous polyposis (FAP) and others. FAP patients express the null phenotype significantly more frequently than do controls (70% versus 40%, $p < 0.0003$). This supports the supposition that carcinogen metabolising genes act as modifiers of phenotypic expression in FAP patients, as is the case in the animal model for FAP.² If this is indeed so in humans, then the concept of FAP as a single gene disorder must be questioned.

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- 1 Debinsky HS, Cottrell S, Phillips RKS, Spigelman AD. GST Mu1 genetic polymorphism in familial adenomatous polyposis (FAP). *Gastroenterology* 1995; 108: A459.

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Urinary excretion of TNF receptors

EDITOR,—We would like to comment on the paper by Dr Hadziselimovic and colleagues (*Gut* 1995; 37: 260–3) investigating urinary excretion of TNF receptors in children with inflammatory bowel disease (IBD). Their findings of increased urinary concentrations of p55 and p75 soluble TNF receptors in patients with active disease is in agreement with studies showing increased systemic concentrations of these receptors in adult patients with Crohn's disease and ulcerative colitis.^{1–3}

Soluble TNF receptors are released in Gram negative sepsis and experimentally in response to endotoxin, formylated oligopeptides, and TNF.⁴ It is unclear whether these soluble TNF receptors act as inhibitors of TNF activity or as carrier molecules for TNF.⁴ The biological implication of increased circulating and urinary TNF receptors in patients with IBD is equally uncertain. In a recent study, administration of the anti-TNF antibody cA2 to 10 patients with Crohn's disease was associated with a normalisation of Crohn's disease activity index scores in eight patients, but no change in the serum concentration of the soluble TNF receptors p55 and p75.⁵

Irrespective of the exact role that soluble TNF receptors have in IBD, measurement of urinary or circulating concentrations of these receptors may prove useful in predicting those patients likely to relapse.

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Reply

EDITOR,—The authors are quite right in their assertion that the biological function of the soluble TNF receptors is still unknown. But for whatever reason, when patients with IBD are in stable remission for at least three months, the concentration of the soluble TNF receptors p55 and p75 in the urine normalises. Although Van Dulleman *et al*¹ reported that there was no change in the serum concentrations of the soluble TNF receptors in their patients, their results cannot be compared with those of our study. Despite

the decreased CDAI scores, which are considered universally as unreliable and subjective assessments, remission induced in these patients by the novel treatment is probably only short lived. The high concentrations of soluble TNF receptors show, in our opinion, that these patients have not achieved a stable remission. We have found that the concentration of the soluble TNF receptors in the urine is a useful prognostic indicator for determining the efficacy of treatment and predicting a change in the status of the disease: relapse and remission.

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Transfusion for variceal bleeding in cirrhotic patients

EDITOR,—We wish to comment on the article by McCormick *et al* (*Gut* 1995; 36: 100-3) suggesting that secondary haemodynamic changes in the splanchnic circulation after variceal bleeding may contribute to increased risk of further haemorrhage. They suggested that the increased splanchnic blood flow could result either from a reflex portal hyperpressure or to overtransfusion during resuscitation. The reflex portal overpressure could be related to neuroendocrine stimulus induced by the presence of blood in the gut. In a previous trial, we have shown that whole gut irrigation with isotonic mannitol reduced blood transfusion requirements and rebleeding rate after variceal bleeding.¹ To investigate the possible deleterious influence of blood transfusions, we have compared, in a pilot randomised study, two protocols of transfusion in cirrhotic patients after recent (<24 hours) and severe variceal bleeding (packed cell volume <27%). In group 1 (n=43), patients were transfused to reach a packed cell volume value of 25±2% and in group 2 (n=47) 32±2%.² Patients were mainly Child-Pugh grades B and C without any difference between the two groups. There were six Child-Pugh A, 20 B, 17 C in group 1 and seven A, 23 B, and 17 C in group 2. In patients with active bleeding, haemostasis was obtained by balloon tamponade. The percentage of patients with active bleeding was similar (52%) in the two groups. No patient received vasoactive drugs. The follow up period was six days after the initial endoscopy performed at admission. Recurrent bleeding was defined by a new bleeding episode, a 3% or more drop of the packed cell volume value or the lack of correction of the initial value of packed cell volume despite transfusions, or all three. At day 6, the mean number of blood units received by the patients was 2.6 in group 1 versus 4.4 in group 2 (p<0.001). The rebleeding rate was 40% in group 1 and 48% in group 2 (NS) and the death rate was 14% in group 1 and 12% in group 2 (NS). Complications (hepato-renal failure, sepsis, hepatic encephalopathy) occurred with a similar frequency in the two groups. In this pilot study, the absence of significant benefit with the low transfusion rate is probably due to a beta

error. Nevertheless, in accordance with McCormick's opinion, it suggests that limited blood transfusions could reduce the early rebleeding rate in cirrhotic patients with variceal bleeding without deleterious effects. A large multicentre trial is actually in progress to compare the results of the two transfusion protocols in cirrhotic patients with variceal bleeding treated by vasoactive drugs and emergency sclerotherapy.

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Gastric metaplasia and *Helicobacter pylori* infection

EDITOR,—Dr Savarino and his colleagues (*Gut* 1995; 37: 445-6 [letter]) are not entirely correct in their reference to our paper published in *Gut* in 1989¹ and subsequent report.² They quote us as 'failing to show any reversal of gastric metaplasia in the duodenum' following medical antisecretory treatment. Although our numbers were small, we reported a noticeable difference between the persistence of metaplasia, as shown by a light and electron microscopy scoring, at the end of one year's maintenance treatment following duodenal ulcer healing with cimetidine 400 mg every night and one year's maintenance treatment with sucralfate 1 g twice daily. Gastric metaplasia was absent in two of 14 and minimal in three of 14 of the cimetidine group compared with four of 11 and four of 11 respectively of the sucralfate group. It was noted that *Helicobacter pylori* was absent in those patients with no metaplasia.

In addition, although both groups at the time of the ulcer healing showed a moderate improvement in the mucosal scoring, at the end of the maintenance year the score in four of 14 of the cimetidine group had reverted to their high initial pretreatment score compared with one of 11 of the sucralfate group.

This suggests that treatment that enhances mucosal protection is more likely to enable the duodenal mucosa to revert to normal than reduction of acid secretion.

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Faecal stream diversion in patients with collagenous colitis

EDITOR,—Veress *et al* have published an interesting paper on the microscopic colitis syndrome (*Gut* 1995; 36: 88-6). Their finding on patient 16 whose increased collagen layer was normal after a temporary loop ileostomy is identical to our previous findings. We have presented experiences of faecal stream diversion in collagenous colitis at both Swedish¹ and Scandinavian² conferences on gastroenterology. Our original report included five of our own operated cases but was later extended with four other cases who underwent surgery in other Swedish hospitals. These four patients were included in our study after written permission had been obtained from the patients' doctors.

Our main conclusion from these nine operated patients was that faecal stream diversion induced clinical and histopathological remission in collagenous colitis.³ After closure of the ileostomy and restoration of intestinal continuity clinical symptoms and the abnormal collagen layer recurred. In a patient who had a sigmoidostomy using the Hartmann procedure, the abnormal collagen layer remained thickened in the proximal colon still exposed to the faecal stream but was normal in the excluded rectosigmoid colon. Later, the sigmoidostomy was replaced by a split ileostomy and at follow up the collagen layer was normal in the whole colon. These findings strongly indicate that a luminal noxious factor may be of pathogenetic importance.¹⁻³ The nature of this luminal factor is unknown. Hypothetically, it may remain in the small bowel after faecal stream diversion, which may explain the early relapse seen in some of the patients after restoration of intestinal continuity.³ Patient 16 in the study by Veress *et al* is the same patient IMB in our report.³

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3 Järnerot G, Tysk C, Bohr J, Eriksson S. Collagenous colitis and faecal stream diversion. *Gastroenterology* 1995; 109: 449-55.

Reply

EDITOR,—Thank you for giving us the opportunity to comment on the letter from Järnerot *et al.* In 1977-1979 one of us (BV) was working together with Dr C Lindström, who first described collagenous colitis.¹ In 1982, we presented our first patient with collagenous colitis at the Meeting of the Swedish Society of Surgeons in Karlstad.² The patient referred to by Järnerot *et al* (no 16) was included in our first joint report from the Central Hospital in Karlstad and Huddinge University Hospital. Järnerot *et al* were unaware that this patient was originally seen by us. The findings described by Järnerot *et al* at the time of the preparation of our paper had only been presented as abstracts.