

## LETTERS TO THE EDITOR

### Survival for colorectal cancer

EDITOR.—The EURO CARE study of Gatta *et al* (*Gut* 2000;47:533-8) provides important information on survival for colorectal cancer in different parts of Europe. In particular, it relates survival to stage, a feature which was absent from earlier studies.<sup>1-3</sup> The study also looks at procedures used in determining stage which may be important factors in accurate staging—for example, higher numbers of lymph nodes sampled will be more likely to detect lymph node metastases and consequent upstaging of tumours.<sup>4</sup>

However, the usefulness of this study is reduced by inaccuracies in the descriptions of staging and choice of staging system. The authors have chosen to use Dukes' staging system which has been in use for at least 50 years and has been well characterised by many studies with long term follow up.<sup>5-9</sup> Unfortunately, they have given incorrect labels to some of the stages in this system. In tables 2 and 3, Dukes' stages A and B are described as being "confined to the bowel wall" which is incorrect. Dukes' stage A is invasion into, but not through, the bowel wall whereas Dukes' stage B is invasion through the bowel wall but without lymph node metastases.<sup>7</sup> Dukes' stages A and B combined could have been correctly labelled as no lymph node metastases. In the methods section the authors state that where only the TNM stage was available, TNM categories were converted to Dukes' stages and where stage was not explicitly stated this was reconstructed from information records (in 45% of cases in the study). It is hoped that the correct descriptions of Dukes' staging were used for this encoding process.

While it is understandable that the authors have chosen to use a well established staging system, the Dukes' system does have a major flaw which is improved by the TNM system.<sup>10</sup> The Dukes' B stage contains tumours which may be in the pT3 stage (tumour invades through the muscularis propria into the subserosa or into non-peritonealised pericolic or perirectal tissues) or pT4 stage (tumour directly invades other organs or structures and/or perforates visceral peritoneum) both with a pN0 nodal stage. The prognosis for pT3 pN0 tumours is relatively good whereas the prognosis for pT4 pN0 tumours is poor with a high risk of local recurrence.<sup>8, 11</sup>

A final histopathology observation on this study concerns the threshold set for the number of lymph nodes examined. The authors state that "it is generally considered that at least 12 lymph nodes should be examined for accurate staging" but this is not a universally accepted threshold. The recommendation from the Royal College of Pathologists in the UK is that all lymph nodes identified in the resection specimen should be examined histologically but does not specify an arbitrary minimum number<sup>12</sup> as it is recognised that the number of lymph nodes identifiable in a specimen varies with a number of factors which include the type of specimen, extent of resection, preoperative chemotherapy and/or radiotherapy, and the

patient's immune response, in addition to the diligence with which the pathologist dissects the specimen. In a recent study in a teaching hospital with optimal dissection and sampling of the entirety of each lymph node the median number of lymph nodes identified was 12<sup>1</sup> so a blanket statement that "at least 12 lymph nodes" should be examined is an unhelpful pressure on hard pressed diagnostic histopathologists.

These criticisms should not obscure the valuable information that is reported in this study but they do suggest that a greater histopathology input at its inception would have been helpful.

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### Reply

EDITOR.—We thank Dr Cross for his interest in our paper (*Gut* 2000;47:533-8). The issue he raised concerning the definition of stage at diagnosis is important for meaningful comparison of cancer statistics between populations. We are pleased to offer the following clarifications.

### INACCURACIES IN STAGE DESCRIPTIONS

We confirm that we incorrectly referred to Dukes' A and B colorectal cases as "confined to the bowel wall." As pointed out by Dr Cross, Dukes' B includes tumours perforating the visceral peritoneum and directly invading other organs (T4), and our category "Dukes' A and B" encompassed T1-T4 N0 M0. A better definition of this category would have been "absence of lymph node and distant metastasis." The stage definitions of Hermanek and Sobin<sup>1</sup> were in fact used for the encoding process, as stated in the article. Furthermore, they were specified a priori in

the study protocol as the reference standard, so as to avoid problems of comparison between registries.

### CHOICE OF STAGING SYSTEM

We used Dukes' instead of TNM because in 1990 Dukes' was the most widely used staging procedure in all cancer registry areas. The other reason we decided to use Dukes' was that from the information available to us (patients' clinical and pathological notes), it was not possible to clearly separate pT3pN0 from pT4pN0 cases (all Dukes' B) which, as Dr Cross pointed out, differ markedly in prognosis. Furthermore, it was often impossible to distinguish pT2 from pT3 cases, and for this reason we considered Dukes' A and B together. The population based nature of our study implied the use of information from numerous hospitals and pathology laboratories, which had various ways of recording information. Greater standardisation of stage reporting in pathology notes would be highly desirable.

We are currently engaged in a new high resolution EURO CARE study on colorectal cancers diagnosed in 1997 when the TNM system was more widely used than in 1990. However, the distinction between pT categories is still not adequately made in a considerable number of cases.

### NUMBER OF LYMPH NODES EXAMINED

Our statement that "at least 12 lymph nodes should be examined for accurate staging" derives from the International Documentation System, referenced by Fielding and colleagues<sup>2</sup> in their article in 1991. This contains the recommendation that, "Before deeming a radical resection to be without lymph node metastasis, it is recommended that at least 12 lymph nodes be examined..." Furthermore, the 1993 TNM supplement<sup>3</sup> states: "histological examination of a regional lymphadenectomy specimen... will ordinarily include 12 regional lymph nodes," and this number is considered "adequate for staging".

Our analysis used four categories for number of nodes examined: 0, 1-5, 6-11, and  $\geq 12$ ; choosing other division points did not change the rank of the registries. However, it is interesting to note that in the hospital study taken as an example by Dr Cross<sup>4</sup>, 12 or more lymph nodes were examined in 50% of cases—well above the percentages reported by our study (range 2-31%). This suggests that in 1990 the extent of resection or the thoroughness of the pathological examination (or both) would not be considered adequate by today's standards.

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### *Helicobacter pylori*, Lewis antigens, and inflammation

EDITOR.—In a recent issue of *Gut*, Zheng *et al* reported that peptic ulcer disease was associated with increased expression of Lewis (Le) antigens but not *cagA*, *iceA*, or *vacA* in Chinese patients infected with *Helicobacter pylori* (*Gut* 2000;47:18–22). These data raise as many questions as they answer and add further controversy to an intriguing area of microbiology and gastroenterology.

Le<sup>x</sup> and/or Le<sup>y</sup> blood group determinants are commonly found on the lipopolysaccharide (LPS) of *H. pylori* isolates. These determinants have been identified on approximately 80–90% of isolates from all patient series examined to date.<sup>1–3</sup> In our own series, 74/84 (88%) isolates expressed either Le<sup>x</sup> or Le<sup>y</sup>, with 47/84 (56%) of isolates co-expressing both determinants.<sup>4</sup> No difference was identified in the prevalence of Le<sup>x</sup> or Le<sup>y</sup> expression on *H. pylori* strains isolated from ulcer patients and those strains isolated from patients with chronic gastritis, nor was an association demonstrated between host and bacterial Lewis phenotype. Since the report of Zheng *et al* we have reviewed our raw data regarding the prevalence of strains expressing two or more Lewis antigens. We found that two or more Le determinants were present in 9/12 (75%) isolates from duodenal ulcer patients and in 4/10 (40%) isolates from gastric ulcer patients. This gives an overall prevalence of 59% (13/22 isolates) for isolates from patients with ulcer disease. Thirty four of 62 (55%) patients with histological evidence of chronic gastritis also had isolates expressing two or more Le antigens.

A significant difference between *H. pylori* strains isolated from Chinese patients and those from our population is the finding of 48/108 (44%) isolates expressing three or more Le antigens compared with 11/84 (13%) isolates from a homogenous Irish population. In our series, only one strain expressed all class 1 and class 2 Le antigens—that is, Le<sup>x</sup>, Le<sup>y</sup>, and their isomers Le<sup>z</sup> and Le<sup>z</sup>. This unusually high prevalence of three or more antigenic determinants raises the question of whether or not these differences were due to strain variation or relate to antibody specificity. Zheng *et al* also suggest that blood group A determinants have not been identified on *H. pylori* isolates. We have previously described this determinant on a *H. pylori* strain expressing both Le<sup>x</sup> and Le<sup>y</sup>.<sup>4,5</sup> However, the role of fucosylated blood group determinants such as blood group A, B, or H type-1 on *H. pylori* LPS is as yet undetermined. This contrasts with the well established role of blood group A in *H. mustelae* mediated gastric autoimmunity in the ferret model of gastric autoimmunity.<sup>6</sup>

These observations on the role of expression of multiple Le determinants are interesting, given the nature of our study population where patients with ulcerative disease made up only 26% of the overall study population. These individuals were infected with strains with a relatively well conserved pattern of Le determinant expression. Both genotypic conservation and adaptation of *H. pylori* to host populations is well recognised in *H. pylori* infection. Clonality is a recently described property of this bacterium and merits discussion in relation to Le determinants. In recent studies, *H. pylori* strains isolated from 12

human hosts and 16 experimentally infected rodents showed Le expression to be highly uniform in isolates from different rodents infected for up to 20 weeks' duration.<sup>7</sup> Substantial differences in Le expression were found among isolates from human patients and these differences were unrelated to the presence of random amplified polymorphic DNA sequences or *cagA* status. It has been suggested therefore that variation in *H. pylori* Le expression in genetically similar microorganisms in human subjects may provide a pool of bacterial phenotypes for the continuous selection of host adapted populations for persistence.<sup>7</sup> Clonality such as that reported for Le determinants could result in the presence of antibodies against the predominant Le epitope in strains from an individual host in vivo. A previous study has suggested that in mimicking host gastric epithelium, *H. pylori* isolates not only express Le<sup>x</sup> and Le<sup>y</sup>, but that their relative proportion of expression corresponds to the host Le blood group phenotype (secretor status).<sup>3</sup> We and others have found no evidence to support this hypothesis.<sup>4,8</sup>

In Asian populations, where the prevalence of *cagA* genotype is high among *H. pylori* isolates, no association between *cagA* genotype and ulceration has been found.<sup>9</sup> Recent data have demonstrated that the prevalence of *cagA*+ *H. pylori* isolates is 44% among a group of 1025 men from 18 British towns.<sup>10</sup> The consequence of this prevalence to disease in the general population is uncertain. However, given the association between *cagA* and more aggressive pathology among Western populations,<sup>11</sup> the relationship between various virulence factors such as *cagA* and putative modifying or colonisation factors such as Le determinants must be clarified. Other data from our group have provided preliminary evidence that *cagA*, *vacA*, or *vacA* expression does not correlate with the level of Le antigen expression.<sup>12</sup> However, no large dataset is available from a homogenous Western population. The original series that suggested that *cagA* status was related to Le antigen expression had 94 isolates from 19 countries and therefore is subject to marked bias.<sup>2</sup>

Lastly, one issue that requires further clarification is the relationship between bacterial Le antigen expression and neutrophil or lymphocytic infiltrates in the host gastric mucosa. In other work, we have shown that inflammatory responses to *H. pylori* infection may be influenced at least in part by host Le<sup>x</sup> expression.<sup>13</sup> Moreover, we have demonstrated that blood group O non-secretors had a significantly higher grade of lymphocyte infiltration of the gastric mucosa compared with non-O non-secretors.<sup>6</sup> We have also demonstrated that Le<sup>x</sup> expression was associated with increased acute inflammatory infiltrates in patients with either ulcer disease (duodenal and gastric) or chronic gastritis.<sup>4</sup> Based on the high prevalence of both Le<sup>x</sup> and Le<sup>y</sup> expression in Chinese patients, the role of inflammation needs to be explored further.

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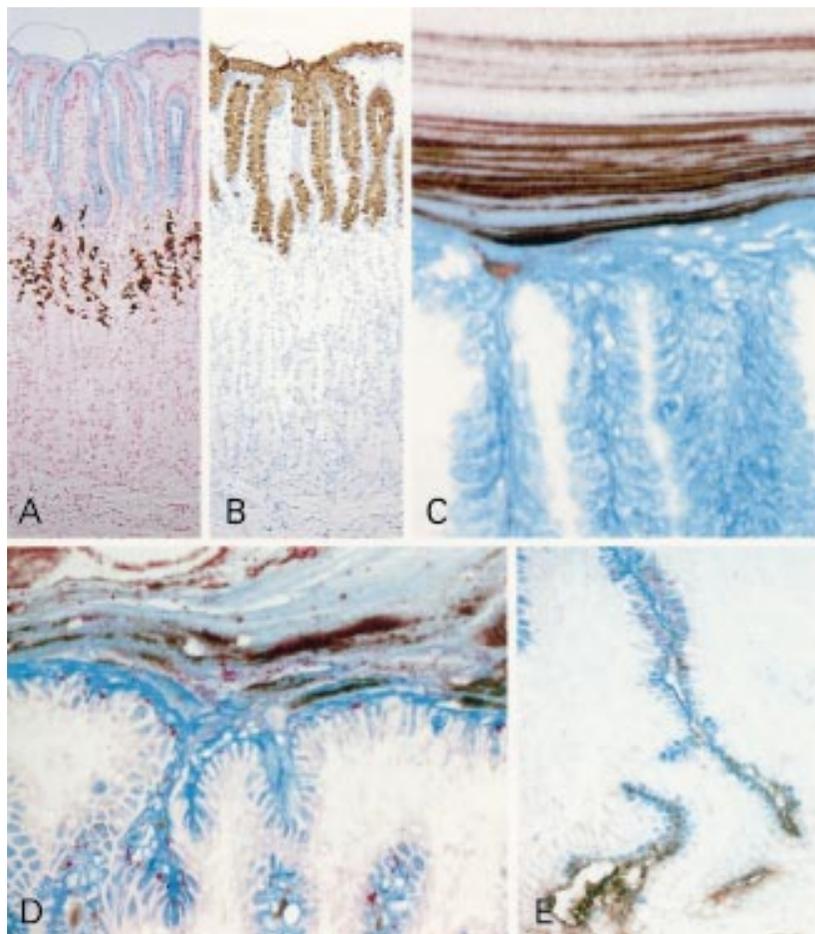
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### Relation of *H. pylori* to gastric mucins and gastric surface mucous gel layer

EDITOR.—We read with considerable interest the excellent article on *Helicobacter pylori* colonisation with MUC5AC in the human stomach by Van den Brink *et al* (*Gut* 2000;46:601–7). The authors used antibodies against gastric mucin core proteins (anti-MUC6 and MUC5AC (45M1)) in conjunction with antibody against *H. pylori* and demonstrated attachment of *H. pylori* to gastric surface mucous cells and the presence of *H. pylori* in extracellular mucins derived from gastric surface mucous cells.

These findings are similar to those previously reported by us<sup>1</sup> using histochemical staining specific for gastric mucins (dual staining consisting of galactose-oxidase cold thionine Schiff reaction (GOTS) and paradoxical concanavalin A staining (PCS))<sup>2</sup>, and immunostaining for *H. pylori*. GOTS recognises galactose or N-acetyl galactosamine residues of gastric surface mucous cell mucins and stains gastric surface mucous cell mucins blue (fig 1A). Histochemical reactivity of GOTS is identical to that of immunostaining with anti-MUC5AC (45M1) (fig 1B). PCS recognises the specific sugar residues with peripheral N-acetyl glucosamine in gastric gland mucous cells (cardiac gland cells, mucous neck cells, and pyloric gland cells) and stains gastric gland mucous cell mucins brown (fig 1A). We used tissue sections from surgically resected stomachs fixed in Carnoy's solution which has the



**Figure 1** (A, B) Formalin fixed normal fundic mucosa. (A) Galactose-oxidase cold thionine Schiff reaction (GOTS) stains gastric surface mucous cell mucins blue and paradoxical concanavalin A staining (PCS) stains mucous neck cell mucins brown (dual staining of GOTS-PCS). (B) Immunostaining with anti-MUC5AC (45M1) labels gastric surface mucous cells. The reactivity of anti-MUC5AC (45M1) is identical to that of GOTS (see (A)) (immunoperoxidase method with anti-MUC5AC (45M1)). (C) Carnoy fixed gastric mucosa without *Helicobacter pylori* infection. The surface mucous gel layer had a characteristic structure consisting of laminating layers of two types of mucins; the surface mucous cell type (blue) and the gland mucous cell type (brown) (dual staining of GOTS-PCS). (D, E) Carnoy fixed gastric mucosa with *H. pylori* infection. (D) *H. pylori* is stained red by an immuno-alkaline phosphatase method with anti-*H. pylori* antibody. Numerous *H. pylori* exist in the surface mucous gel layer and on and between the surface mucous cells. In the surface mucous gel layer, *H. pylori* preferentially colonise in the layer of surface mucous cell type mucins (dual staining of GOTS-PCS followed by immunostaining for *H. pylori*). (E) *H. pylori* is stained red by an immuno-alkaline phosphatase method with anti-*H. pylori* antibody. Numerous *H. pylori* preferentially exist on and between the surface mucous cells but not on pyloric gland cells (dual staining of GOTS-PCS followed by immunostaining for *H. pylori*).

advantage of fixing the gastric surface mucous gel layer (SMGL) in paraffin embedded tissue sections.<sup>3</sup>

Dual staining of GOTS-PCS of Carnoy fixed gastric mucosa without *H. pylori* infection showed that SMGL had a characteristic structure consisting of laminating layers of two types of mucins: the surface mucous cell type and the gland mucous cell type (fig 1C).<sup>1,3</sup> Dual staining of GOTS-PCS in conjunction with immunostaining for *H. pylori* in Carnoy fixed stomachs with *H. pylori* infection revealed that *H. pylori* characteristically existed on and between the surface mucous cells and in the SMGL (fig 1D).<sup>1</sup> In the SMGL, this organism was most often associated with the layer of surface mucous cell type mucins (fig 1D, E).<sup>1</sup> Receptors responsible for adherence of *H. pylori* might exist only on the plasma membrane of surface mucous cells. In the SMGL, these receptors appear to be mixed with the surface mucous cell type mucins. The SMGL inhabited by *H. pylori* revealed marked derangement of the

multilaminating structure with fragmentation of the mucin layers and formation of vacuoles (fig 1D, E).<sup>1</sup> After eradication of *H. pylori*, the SMGL regained the laminating structure.<sup>1</sup> Alteration of the SMGL found in *H. pylori* infected stomachs suggests destruction of the SMGL by bacterial lipase and protease from *H. pylori*.<sup>4,5</sup>

*H. pylori* in the SMGL was more abundant than that attached to the surface mucous cells (fig 1D, E).<sup>1</sup> The SMGL appears to be the major site of *H. pylori* colonisation and may serve as a vehicle for diffusion of *H. pylori* to other sites in the stomach and duodenum.

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## Reply

EDITOR,—I thank Dr Ota *et al* for their interest in our work. I was already familiar with the exquisite pictures of the laminated structure of gastric mucus by these authors. The present new data as shown, in conjunction with our previous data, shed more light on the phenomenon of *Helicobacter pylori* attachment in the stomach. Although I generally support the conclusions of Ota *et al*, the following remarks are in order.

It seems very likely from figure 1A and 1B of their letter that the surface mucous cells that produce MUC5AC (by 45M1 immunostaining) were stained blue on galactose-oxidase cold thionine Schiff reaction (GOTS) staining. This implies that the blue sublayers within the laminated extracellular mucus would also be composed of MUC5AC. Also, as the authors show in fig 1 (D, E), *H. pylori* was specifically localised to: (1) the blue layers within the mucus layer (that is, most likely MUC5AC; fig 1D); and (2) the blue stained cells (that is, most likely producing MUC5AC; fig 1D, E). Implicit in our collective data is that the brown staining layers, as demonstrated by paradoxical concanavalin A staining (PCS), are probably composed of MUC6. Collectively, these data corroborate our previous conclusion that MUC5AC and MUC5AC producing cells (and not MUC6) are the main attachment sites for *H. pylori* in the stomach. Yet final prove awaits immunohistochemical co-localisation of MUC5AC, MUC6, and *H. pylori* in the mucus layer. As Carnoy's solution may not allow simultaneous immunochemical detection of these components, technical problems presently hamper final conclusions. In pursuing this, I think it is extremely important to concentrate on the primary gene products—that is, localisation of MUC5AC and MUC6 proteins—rather than on carbohydrates on complex

molecules. Glycosylation is a complex process that may easily become disturbed in *H pylori* infection, for example. Thus as glycosylation may change under pathological conditions, I would urge the authors and other workers in the field to concentrate on the invariant part of the mucins: the non-O-glycosylated parts of the protein, as discussed by us previously.<sup>1</sup>

One of the issues addressed by Ota *et al* is the correlation of *H pylori* infection with disturbance of the laminated gastric mucus layer, as demonstrated in fig 1C versus fig 1D. In common with the authors, it is tempting to speculate that *H pylori* has a direct role in this disturbance. Similar to most bacteria, *H pylori* produces enzymes that in principle can degrade mucins, such as proteases and "mucinases", as referred to by the authors. I personally doubt whether *H pylori* is directly responsible for disturbance of the mucus layer for two reasons. Firstly, as the bacterium resides in the mucus layer, what would be the benefit of destroying its own milieu? Secondly, there are data by many authors to indicate that the tissue dynamics of the gastric mucosa is altered by *H pylori* infection. Accompanying the inflammatory response, epithelial turnover is enhanced, and there are profound shifts in cell populations within the epithelium. As a result, which was also mentioned in our previous *Gut* article, MUC6 may be over expressed in *H pylori* infected stomach at the expense of MUC5AC expression. From our own work, and that of Byrd and colleagues,<sup>2</sup> it appears that the number of cells expressing MUC5AC declines and that concomitantly the relative number of MUC6 producing cells rises. Meanwhile, we have further data to corroborate this notion, and it appears that there is a statistically highly significant decrease in MUC5AC producing cells (Van de Bovenkamp JHB, Korteland-Van Male A, Büller HA, *et al*, unpublished data). Thus shifts in expression levels of individual mucins that constitute the mucus layer may easily explain the disturbance of the gastric mucus layer. Obviously, this type of discussion calls for a more dynamic approach to this problem. Only a detailed study of mucin biosynthesis (at the mRNA and protein levels and rate of secretion) may reveal the truth behind my speculations. At this point we will have to make do with an educated guess.

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### *Helicobacter pylori* infection and acid secretion in patients with duodenal ulcer in Japan

EDITOR.—*Helicobacter pylori* infection affects gastric acid secretion of the host in various ways. For example, corpus gastritis and subsequent development of mucosal atrophy induced by *H pylori* infection result in decreased acid secretion.<sup>1</sup> In contrast, several investigators have reported elevated acid secretion in patients with duodenal ulcer

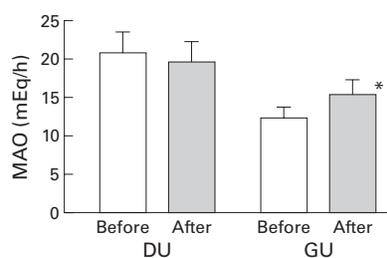


Figure 1 Effects of *Helicobacter pylori* eradication on maximum acid output (MAO) in Japanese patients with duodenal (DU) and gastric (GU) ulcers. MAO in response to pentagastrin (6 µg) was measured before and six months after *H pylori* eradication. \* $p < 0.05$  *v* before eradication.

(DU) which is decreased by *H pylori* eradication with simultaneous reduction of serum gastrin.<sup>2,3</sup> Supporting these data, Iijima *et al* (*Gut* 2000;46:20-6) reported that eradication of *H pylori* was accompanied by an increase in acid secretion in patients with gastric ulcer (GU) in whom corpus gastritis and/or atrophy are frequently observed, whereas acid secretion decreased after eradication in those with DU. However, in countries such as Japan where corpus gastritis and mucosal atrophy are common among patients infected with *H pylori*, the situation is not as simple.

We performed a similar study by measuring basal acid output (BAO) and maximum acid output (MAO) using pentagastrin administration before and six months after eradication in Japanese patients with GU (n=21) and DU (n=22). In both patients with GU and DU, serum gastrin levels were significantly higher than those of control subjects ( $p < 0.01$ ) before eradication; they decreased significantly after eradication (GU: 129.7 (19.2) to 94.6 (15.0) pg/ml; DU: 116 (19.8) to 90.3 (16.3) pg/ml;  $p < 0.05$ ). These data are similar to those of Iijima *et al* (*Gut* 2000;46:20-6). In contrast with their data however we could not find any significant changes in MAO after eradication in DU patients although *H pylori* eradication resulted in a significant increase in MAO in patients with GU (fig 1). Indeed, as shown in fig 2, changes in MAO varied from patient to patient among DU individuals. Subsequently, we attempted to elucidate the

relationship between changes in serum gastrin and MAO more precisely. As expected, in patients with GU, a positive correlation was found between the decrease in serum gastrin and increase in MAO following *H pylori* eradication (fig 2). These data suggest that recovery of acid secretion by *H pylori* eradication in patients with GU is responsible for the decrease in serum gastrin levels. In contrast, in patients with DU, no significant correlation was observed between changes in serum gastrin and acid secretion. When we analysed the DU data more precisely however, we found an interesting fact: acid secretion of one group of patients (group C) increased with a simultaneous decrease in serum gastrin. These changes in response to *H pylori* eradication were similar to those observed in GU patients. Therefore, we divided DU patients into three groups according to changes in MAO by *H pylori* eradication (group A: MAO decreased more than 1 mEq/h; group B: no change; group C: MAO increased more than 1 mEq/h) and found that group C patients had the lowest serum pepsinogen (PG) I levels and PG I/II ratios among the three groups (PG I: group C 44.7 (1.6), group A 64.5 (2.5), group B 57.8 (2.6); PG I/II: group C 2.52 (0.18), group A 3.98 (0.24), group B 3.60 (0.19);  $p < 0.05$ ). These data indicate that patients in group C had suffered atrophy of the fundic mucosa.

Data on changes in gastrin stimulated acid secretion after eradication in patients with DU are controversial,<sup>2,5</sup> although most reports show a decrease in acid secretion.<sup>2,3</sup> As Iijima *et al* indicated, the most likely reason for the controversy may be that a considerable number of patients with DU had corpus gastritis, which may somehow cause hypoacidity. We previously reported that gastric acid secretion in Japanese subjects is lower than that in Europeans or North Americans, irrespective of *H pylori* infection.<sup>6</sup> As *H pylori* is reported to induce corpus gastritis more easily in subjects with decreased acid secretion,<sup>7,8</sup> we suggested that innate low gastric acid secretion of the Japanese may be responsible for the higher incidence of corpus gastritis and atrophy in Japanese subjects with *H pylori* infection. This appears to be the case even for patients with DU in Japan, and it may be the reason why acid secretion was not significantly reduced after *H pylori* eradication in our DU patients.

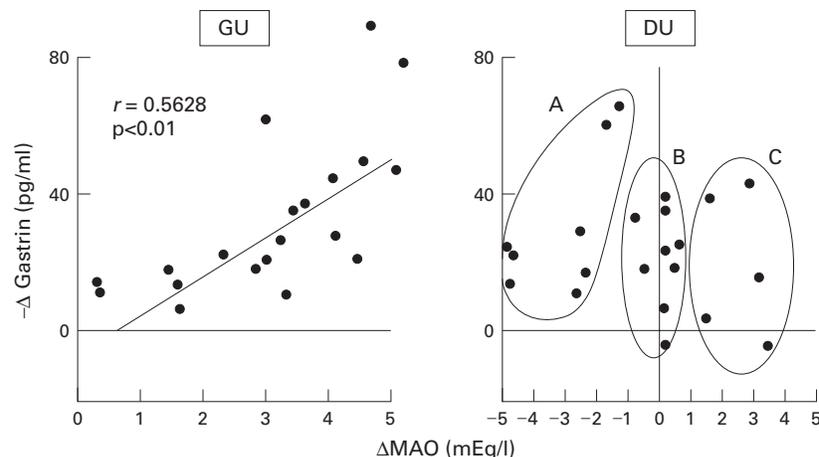


Figure 2 Correlation between decrease in serum gastrin concentration ( $-\Delta$ Gastrin) and increase in maximum acid output ( $\Delta$ MAO) in response to pentagastrin (6 µg) by *Helicobacter pylori* eradication in Japanese patients with duodenal (DU) and gastric (GU) ulcers. Groups A, B, and C show DU patients in whom MAO decreased (more than 1 mEq/h), did not change, and increased (more than 1 mEq/h) after eradication, respectively.

It has been a matter of debate whether gastric acid hypersecretion observed in patients with DU is a result of *H pylori* infection or if the infection accelerates development of DU in subjects who originally had acid hypersecretion. Our data showing that acid secretion was recovered in association with the decrease in serum gastrin levels in some DU patients and that MAO levels in patients with DU after eradication are still higher than those of normal subjects without *H pylori* infection may support the latter idea. Moreover, this idea may explain the fact that there are more patients with GU than DU among Japanese subjects,<sup>9</sup> who exhibit lower acid secretion than Western patients.<sup>6</sup>

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## BOOK REVIEWS

**Colon Microbiota, Nutrition and Health.** Edited by G R Gibson, M B Roberfroid (Pp 304; illustrated; £93.00). The Netherlands: Kluwer Academic Publishers, 1999. ISBN 0412798808

I was taught as a medical student that the major function of the colon was that of a storage organ. Since then premises about the colon have evolved and the complexities of colon function is much better understood, as

described by Gibson and Roberfroid's (editors) multi-authored book *Colon Microbiota, Nutrition and Health*.

Although the editors state that the purpose of the book is to overview current knowledge of the activities and functions of the gut microflora, the scope goes beyond these boundaries and takes us on an ecological journey into the exciting life of gut microflora and their impact on colon function in health and disease, and the intimate critical relationship between diet, bacteria, and quality of life.

Gastroenterologists are still recovering from the impact that a bacterium, *Helicobacter pylori*, has had on upper gastrointestinal tract pathology. In this context it is interesting to note that the large bowel is the most heavily colonised part of the gastrointestinal tract yielding up to 10<sup>12</sup> bacteria per gram of intestinal contents in healthy human subjects. It is a complex ecosystem in which the numerous and different species of bacteria degrade and ferment substrates that have escaped digestion in the small bowel. Major genera include bacteroides, bifidobacteria, lactobacilli, clostridia, and enterobacteria, and the main products of bacterial fermentation of the substrate that reaches the colon are short chain fatty acids (SCFA) and gases, including hydrogen, carbon dioxide, and in some individuals, methane. The relevance of SCFA is that they act as a source of energy for intestinal mucosal cells and reduce the pH of colonic contents. One particular SCFA, butyrate, may be important in protecting against colorectal cancer. The relevance of fermentation to human metabolism can be gauged from the fact that the energy equivalent of 15–40 g of carbohydrate is metabolised by the large bowel.

The book outlines the technological revolution that has occurred in understanding the natural microbial world. Molecular biology has invaded gut microbiology with the limitations of enrichment cultural techniques being integrated into techniques based on the detection of genomic DNA or analysis of rRNA.

A fascinating aspect of the book that concerns and affects all of us are the chapters on food. With the craze of low carbohydrate diets to counter obesity sweeping the USA, it is refreshing to realise the importance of carbohydrates and the concept of functional food. These foods target specific functions in the body in a positive way due to the presence of health enhancing ingredients. Colonic foods are an example of such functional foods that target the large intestine. These are foods that contain an ingredient that does not undergo significant modification during transit through the small intestine but reach the colon where they are utilised by the resident bacteria producing metabolites that influence the physiological and biochemical processes in a beneficial manner. Dietary fibre is the best known of the "colonic foods" and is divided into soluble and insoluble fibre. Soluble fibres include pectin, guar gum, B glucan, and psyllium, and result in modest reductions in blood lipids affecting total and LDL cholesterol fractions. Insoluble fibres (cellulose and lignin) are mainly responsible for faecal bulking. Dietary fibre may play a protective role in diverticular disease and colorectal cancer. Other functional foods are the fructans and resistant starch which, in animal models, affect triglyceride rich fractions. A novel and potentially important approach to prevention and therapy of

colonic diseases is the concept of prebiotics and probiotics. The probiotic approach involves adding live microorganisms to the gastrointestinal tract while prebiotics enhance certain components of the existing flora. Probiotics have potential in the prevention and treatment of rotavirus infections, lactose malabsorption, and food allergy. Tentative claims for benefits of prebiotics include reduction in obesity, improved control of non-insulin dependent diabetes, reduction in the risk of atherosclerotic cardiovascular disease, and prophylaxis of acute gastroenteritis.

How does the above affect individuals? It seems that we should include the following foods in our diet: garlic, onions, asparagus, chicory, dandelion, artichokes, soy beans, leeks, Jerusalem artichokes, wheat, bananas, and rye. Quite a tall order!

The authors have made an important contribution to the concept of local and systemic effects of the colon and outlined the benefits of a healthy colon and the involvement of the idea that functional foods have prophylactic and therapeutic properties. A minor criticism is that there is repetition of ideas in certain chapters. I would highly recommend this excellent work for gastroenterologists as a seminal study. This book should be used as a guide not only for gastroenterologists, primary care physicians, and nutritionists but also for all health workers.

I SEGAL

**Advances in Inflammatory Bowel Diseases (Falk Symposium 106).** Edited by P Rutgeerts, J-F Colombel, SB Hanauer, *et al* (Pp 318). The Netherlands: Kluwer Academic Publishers, 1999. ISBN 0 7923 8750 3.

The proceedings of medical and scientific meetings, when published, tend to be viewed more as trophies for the participants rather than referenced texts to be consulted at a later date. In this information age, such publications are seldom a first choice for those seeking specific information on selected topics and, even then, the readership is vanishingly small. The present text is therefore a surprise because it offers an excellent overview of recent advances in inflammatory bowel disease (IBD), and blends insights into pathogenetic mechanisms with new therapeutic approaches. As with most Falk symposia, the selection of authors is truly international and each is a leader in the field. Several of the chapters provide more than a review, and are actually quite useful in translating research information into clinical implications of practical value. This is particularly the case in chapters dealing with genetics, cytokines, steroid therapy, and cancer in IBD. The standard of writing is not uniform and, although six editors are listed, I doubt if any had significant editorial input to the chapters. The quality of the book relies therefore on the expertise of the authors which is impressive. What is remarkable is that such a large amount of information can be presented in a concise fashion in such a slim volume and in such a readable manner. I rarely recommend books of this nature for general readership but anyone seeking a concise pain free update and overview of the field would not go far wrong with this text.

F SHANAHAN

## CORRECTION

An error occurred in abstract P98 (*Gut* 2000;47(Suppl III):A72). The correct abstract is published here.

**P98** AN INVESTIGATION INTO THE IMPACT OF ALGINATES AND EPIDERMAL GROWTH FACTOR ON ENDOCYTOSIS-A STUDY IN FOUR OESOPHAGEAL CELL LINES

P.M. McPherson<sup>1</sup>, P.E. Ross<sup>1</sup>, P.W. Dettmar<sup>2</sup>. *Gastroenterology Research Laboratory, Molecular and Cellular Pathology, University of Dundee.* <sup>3</sup>Reckitt Benckiser Healthcare (UK) Ltd, Hull, UK.

**Introduction:** Endocytosis is a process whereby eukaryotic cells take up extracellular material by a variety of different mechanisms. These endocytic functions are of great importance and are involved in the regulation of cell surface receptor expression, maintenance of cell polarity, cholesterol homeostasis and a host of other physiological processes. In this investigation we looked specifically at fluid phase endocytosis and the impact alginates and epidermal growth factor (EGF) have on this activity.

**Background:** Alginates are extracted from seaweed with their structure and properties related to the species of seaweed. They are carbohydrate polymers made up of D-mannuronic (M block) and L-guluronic (G block) acid residues, and may also be made up of

sequences of mixed residues (MG blocks). These carbohydrate polymers appear to promote migration and restitution in gastrointestinal epithelial cells *in vitro* and *in vivo* by modulating the expression and functional activity of cell junctional proteins such as the E-cadherin-catenin complex. EGF is a 6kd polypeptide that has a role in tissue repair, cell proliferation, ulcer healing and cell migration. EGF also inhibits acid production and imparts a cytoprotective mechanism protecting the oesophageal mucosa from gastric refluxate. Similar biological effects have been recognised with alginates that are used extensively in medications to alleviate symptoms associated with gastric reflux

**Methods:** In this study we have used four oesophageal carcinoma cell lines, 2 squamous cell carcinomas and two adenocarcinomas. Cells were incubated with combinations of fluorescent microspheres (0.02µm), alginate and EGF for 1 hour, and then analysed by FACScan®. Alginates were used at a concentration of 2mg/ml and EGF at 10ng/ml.

**Results:**

- All alginates used in this study up-regulate fluid phase endocytosis.
- EGF up-regulates endocytosis.
- Incubation with EGF and alginate up regulates fluid phase endocytosis.
- Levels of up-regulation varied depending on alginate used.
- Alginates up-regulate fluid phase endocytosis more than physiological levels of EGF.

**Conclusions:** We have shown that both alginates and EGF up-regulate fluid phase endocytosis in all cell lines used in this study. However alginates up-regulate this process significantly whereas EGF does not. The mechanism for this alginate action is not yet identified, but it is possible that alginates interact with the receptor for EGF.

## NOTES

### Summer Abdominal Imaging Conference

A five day course designed for the practising radiologist with a primary interest in abdominal imaging, emphasising the most recent advances in helical CT, MRI, US, and gastrointestinal imaging. It will be held on 23–27 July 2001 in Banff Springs, Canadian Rockies. Twenty-five category 1 credit hours. Further information: Janice Ford Benner, University of Pennsylvania Medical Center (Radiology), 3400 Spruce Street, 1 Silverstein Building, Philadelphia, PA 19104, USA. Tel: +1 215 662 6904; fax: +1 215 349 5925.

### Postgraduate Gastroenterology

A course designed for consultants and registrars, including those who do not specialise in gastroenterology, will be held on 9–12 September 2001 in Oxford, UK. Further information: Professor DP Jewell, Gastroenterology Unit, Radcliffe Infirmary, Woodstock Road, Oxford OX2 6HE. Tel: +44 (0)1865 224829; fax: +44 (0)1865 790792; email: derek.jewell@ndm.ox.ac.uk; website: www.medicine.ox.ac.uk/gastro/gastrocourse.htm

### British Association for the Study of the Liver

The 2001 BASL meeting will be held on 13–14 September in London, UK. Further information: Jackie Carter, Centre for Liver Research, University of Newcastle, Floor 4,

William Leech Building, Medical School, Framlington Place, Newcastle upon Tyne NE2 4HH, UK. Tel: +44 (0)191 222 5640; fax: +44 (0)191 222 0723; email: j.a.carter@ncl.ac.uk

### Torino-Toronto First Joined Workshop on Therapeutic Endoscopy

This workshop will be held on 13–15 September 2001 in Turin, Italy. Further information: Anna Botto, MAF Servizi, Congress Division, Via GB Vico, 7, 10128 Turin, Italy. Tel: +39 011 505 900; fax: +39 011 505 976; email: abotto@mafservizi.it

### EACP Scientific and Second Annual General Meeting

The European Association of Coloproctology will hold this meeting on 14–15 September 2001. Further information: Lindsey Whitehouse, Integrity International Event Services, Conference House, 152 Morrison Street, Edinburgh EH3 8EB, UK. Tel: +44 (0)131 200 6055; fax: +44 (0)131 476 4646; email: enquiries@integrity-events.com; website: www.eacp.org

### Asia Pacific Digestive Disease Week

The inaugural APDW will be held on 23–27 September 2001 in Sydney, Australia. This meeting will include a live endoscopy workshop, a wide range of other workshops, and a comprehensive scientific programme including original research and clinical symposia. Further information: Conference Secretariat, Gastroenterological Society of Australia, 145 Macquarie Street, NSW 2000, Australia. Tel: +61 (0)2 9256 5454; fax: +61 (0)2 9241 4586; website: www.gesa.org.au

### 9th Asian Conference on Diarrheal Diseases and Nutrition

This meeting will be held on 28–30 September 2001 in New Delhi, India. The organisers hope the meeting will promote meaningful and effective collaboration among individuals/institutions towards control of the major health problems in Asia, particularly those affecting women and children. Further information: Professor M K Bhan, Coordinator, Centre for Diarrheal Disease and Nutrition Research, All India Institute of Medical Sciences, New Delhi. Tel: +91 11 6963822; fax: +91 11 6862662; email: ascodd2001@rediffmail.com

## RETRACTION

The authors of abstract number 071 of the BSG Annual Meeting abstract book (*Gut* 2000;48(suppl I):A20) would like to publish a retraction. This is due to the discovery of an error in the data presented which changes the conclusions of the abstract. The potential error was of a technical nature which the authors were unable to resolve until the return of specific technical support to the laboratory.

The authors have found that their genotyping of the IL-10 polymorphism is inverted on what was presented in the abstract, meaning that the association of ulcerative colitis is with the high producing IL-10 allele. They were only recently able to confirm this by direct DNA sequencing.

The authors would like to apologise for the error.