The secretion of acid is an important function of the human stomach. In humans, although the rate of secretion varies, acid is continuously secreted by the gastric mucosa. During periods of fasting, the rate of acid secretion is low but sufficient to maintain an intragastric pH below 2. The regulation of gastric acid secretion is achieved by the interplay between two major gastric endocrine cells: the gastrin G cell and the somatostatin D cell. Regulation of these cells occurs via stimulatory or inhibitory paracrine, endocrine, and neural pathways. When food enters the stomach, the protein component stimulates G cells situated in the antral region of the stomach to release the hormone gastrin, which stimulates the enterochromaffin-like cells to release histamine and stimulates parietal cells to secrete acid. As the acidity of the stomach and duodenum increases, protective feedback pathways are activated to inhibit further acid secretion. One important acid mediated inhibitory control is related to the release of somatostatin by D cells. Somatostatin exerts paracrine inhibitory control on gastrin release by the antral G cells. The regulation of gastric acid secretion is achieved by the interplay between two major gastric endocrine cells: the gastrin G cell and the somatostatin D cell.}

Helicobacter pylori infection interferes with these physiological control processes, resulting in alterations of gastric acid secretion. Several clinical and animal studies have been performed to examine the effect of H pylori infection on the regulation of gastric acid secretion. Helicobacter pylori is associated with increases in both fasting and meal stimulated gastrin values. In addition, the normal physiological negative feedback control of secretion is impaired. These phenomena are reversible—after H pylori eradication, gastrin concentrations normalise and normal feedback control of gastrin secretion is restored. Therefore, the purpose of our study was to investigate the relation between H pylori status, the type and severity of gastric inflammation, and the location and number of G and D cells in the gastric mucosa. These studies were designed to characterise and clarify the mechanism of altered acid secretion as a result of H pylori infection.
Histological examination

Specimens were fixed in 10% formalin and routinely processed. Paraffin wax embedded sections were cut into 3–4 μm thick serial sections and stained with haematoxylin and eosin. Giemsa staining was routinely performed to detect the presence or absence of *H pylori*. The sections were interpreted by two experienced pathologists (TK and YL), who were unaware of the clinical and endoscopic findings.

The following four parameters were evaluated and graded, as suggested by the Sydney system: (1) chronic inflammation, which is scored on the basis of the chronic inflammatory infiltrate in the lamina propria; (2) active component of inflammation through assessing the polymorphonuclear leucocytes (neutrophilic infiltration); (3) atrophy, which is scored on the basis of the proportional loss of the specialised gastric glands, with or without replacement of intestinal metaplastic tissue; areas with lymphoid follicles, dense infiltration, or both were not graded for atrophy; and (4) intestinal metaplasia, which was scored as absent or occupying less than one third, more than one third, or more than two thirds of the mucosa present. For both antrum and corpus, biopsies were assessed semiquantitatively by a score in each Giemsa stained specimen was identified as a G, D, or endocrine cell type if a dark brown granular reaction was produced by the ABC method. Cells that stained positive for gastrin G cells, somatostatin D cells, and chromogranin by the avidin–biotin complex (ABC) method (DakoCorp, Copenhagen, Denmark). In brief, sections were dewaxed and rehydrated in graded alcohols. Endogenous peroxidase activity was quenched, antigen retrieval was performed by heating for 10 minutes at 100°C in 0.01M sodium citrate, and non-specific staining was reduced by a blocking step. The rabbit antibodies against gastrin (1/1000 dilution), somatostatin (1/1600 dilution), and chromogranin (1/1000 dilution) were applied in phosphate buffered saline containing 1% bovine serum albumin and 0.1% Triton and incubated overnight at 4°C (all three antibodies were from DakoCorp). The following day, a three step detection method was used as described previously, using a biotinylated goat antirabbit immunoglobulin antibody (Dako: 1/500 dilution). Detection was performed with horseradish peroxidase conjugated ABC (Dako) for 60 minutes and peroxidase activity was detected with diaminobenzidine (fast DAB; Sigma, St Louis, Missouri, USA), according to the manufacturer’s instructions, resulting in the formation of a brown reaction product. Finally, the sections were briefly counterstained with haematoxylin, dehydrated in graded alcohols, and mounted. Further controls consisted of omitting the primary and secondary antibodies and the use of an appropriate immunoglobulin control.

Only sections showing the entire axis from the superficial luminal epithelium to the muscularis mucosae were examined. Cells that stained positive for gastrin G cells, somatostatin D cells, and chromogranin endocrine cells were counted in a minimum of three high power fields for each specimen, without reference to the clinical histories. Each cell was identified as a G, D, or endocrine cell type if a dark brown granular reaction was produced by the ABC method. The numbers of endocrine cells were evaluated by two pathologists (TK and YL). Ten well oriented vertical glands in each field were counted. The results were expressed as the total number of cells counted/10 adjacent glands. If the glandular structure was not well oriented, we counted two areas (the area with the most and the area with the least intensive 10 adjacent glands) and calculated the mean. The average number of positively counted cells for each 10
vertical glands was compared between groups. Moreover, the distribution of the cells was also reported (even or uneven).

**Statistical analyses**

Data were analysed using the SPSS statistical package. The χ² test was used to calculate the difference between G and D cells with respect to *H pylori* status, the Mann-Whitney U test for analysing the difference in the grading of gastritis features, and the Spearman rank correlation test for analysing the correlation between different gastritis features and *H pylori* status, and between G and D cells. A p value less than 0.05 was considered to be significant.

**RESULTS**

In total, 122 of the 194 patients who underwent endoscopy for dyspeptic complaints were used in our analyses. Table 1 shows the characteristics of the patients studied. The age and sex distribution did not differ significantly among the different groups.

**Helicobacter pylori status and histopathological changes**

*Helicobacter pylori* infection was found in 58 patients (58 of 122; 47.5%) with both procedures (CLO and Giemsa staining), with the remaining 64 individuals being *H pylori* negative (64 of 122; 52.5%). Among the non-infected individuals, 47 had no histological changes, and 17 had features of chronic gastritis.

The histological scores for chronic inflammation, activity, atrophy, and intestinal metaplasia were higher in *H pylori* positive than in *H pylori* negative patients (*p < 0.05*) and *H pylori* infection was significantly associated with increased antral inflammation and activity (*p < 0.01*). Mucosal atrophy was more prevalent in patients with *H pylori* infection than those without. Antral atrophy was more pronounced than corpus atrophy (*p < 0.01*).

### Table 2  Density of the endocrine cells, gastrin, and somatostatin in *Helicobacter pylori* infected patients, non-infected patients, and normal subjects

<table>
<thead>
<tr>
<th>Status</th>
<th>No of subjects</th>
<th>Chromogranin cell density</th>
<th>G cell density</th>
<th>D cell density</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Antrum</td>
<td>Corpus</td>
<td>Antrum</td>
</tr>
<tr>
<td><em>H pylori</em> (+)</td>
<td>58</td>
<td>49.1 (21)</td>
<td>22.2 (15)</td>
<td>34.4 (19)*</td>
</tr>
<tr>
<td><em>H pylori</em> (−)</td>
<td></td>
<td>49.0 (19)</td>
<td>28.2 (16)</td>
<td>23.1 (10)</td>
</tr>
<tr>
<td>Chronic gastritis</td>
<td>17</td>
<td>49.0 (19)</td>
<td>28.2 (16)</td>
<td>23.1 (10)</td>
</tr>
<tr>
<td>Normal (no gastritis)</td>
<td>47</td>
<td>50.5 (22)</td>
<td>19.1 (9)</td>
<td>26.3 (13)</td>
</tr>
</tbody>
</table>

*Cell density values are mean (SD).*

| * Significant difference (*p*<0.01).

**Expression of gastrin, somatostatin, and chromogranin**

There was no immunoreactivity when the primary antibodies were omitted from the staining procedure. The staining patterns for gastrin, somatostatin, and chromogranin were similar. Positive cells revealed cytoplasmic staining. Most of the positive cells were located in the middle third of the gastric glands, with few in the upper or deeper parts (figs 1, 2). However in *H pylori* positive patients, the distribution of chromogranin and gastrin positive cells moved slightly upwards to just beneath the foveolar gastric pit. G cells were only present in the antrum, whereas D cells and chromogranin positive cells were expressed both in the antrum and the corpus.

The *H pylori* infected individuals had significantly higher numbers of immunoreactive G cells and lower numbers of immunoreactive D cells than both those with non-infected gastritis and those with histological normal gastric mucosa. Significant differences existed between infected and non-infected individuals or normal groups both for immunoreactive G cells and D cells (table 2). A significant correlation was found between antrum immunoreactive G cells and D cells (correlation coefficient, 0.29) (*p < 0.01*).

Figure 3 compares the density of immunoreactive G cells and D cells in each group. The number of immunoreactive G cells was highest among *H pylori* infected patients and lowest among those with normal gastric mucosa. In contrast, the lowest score for immunoreactive D cells was seen in *H pylori* infected patients. The mean density of immunoreactive G cells and immunoreactive D cells in patients with *H pylori* negative chronic gastritis and with normal mucosa were not significantly different. Figure 4 summarises the density of immunoreactive G cells and D cells in relation to the density of *H pylori*. Although the number of immunoreactive G cells was higher and the number of D cells lower in those with *H pylori* infection compared with the *H pylori* negative group,
there was no evidence of a linear relation between H pylori density and the density of either immunoreactive G or D cells. In contrast, the density of immunoreactive G and D cells was similar among those without inflammation and those with minimal inflammation (grade 1) (fig 5). Immunoreactive G cell density increased and D cell density decreased among those with moderate or severe inflammation (fig 5).

Percentage of G and D cells in the stomach
In addition to gastrin and somatostatin, chromogranin was used to identify all endocrine cells. The mean (SD) number of chromogranin positive cells was 49.1 (21) in H pylori infected patients, 49.0 (19) in non-infected gastritis patients, and 50.5 (22) in those with normal mucosa (p > 0.05).

The percentage of G cells and D cells of all endocrine cells in the stomach was 52.0% and 17.5% in normal individuals, and 47.1% and 15% in the H pylori negative group. However in the H pylori infected subjects, 70.1% of chromogranin positive cells were G cells and only 4.9% were D cells. The difference between H pylori infected and non-infected or normal individuals was significant (p < 0.01; table 3).

DISCUSSION
Gastrin G and somatostatin D cells are the major endocrine cells in the stomach known to play an important role in acid secretion. Normally, about 50% of the endocrine cell population of the antrum is made up of G cells and 15% of D cells. In the corpus mucosa, enterochromaffin-like cells form a major proportion of the endocrine cells. However, no study has been carried out to define the exact percentages of these endocrine cells. In our study, approximately 52% of all the endocrine cells in the antrum were G cells and 17.5% were D cells. In the corpus, 8.3% of cells were D cells and no immunoreactive G cells were found. These results are comparable to other studies. We also confirmed that the percentages of G and D cells were altered in H pylori infected gastric mucosa, with an increase in the proportion of G cells and a decrease in D cells.

Gastrin is released from G cells in the gastric antrum and acts via the circulation to stimulate acid secretion. Both H pylori infection and acid secretion are related to the development of duodenal ulcers, prompting several investigators to examine the effect of H pylori infection on the control of acid secretion. In 1989, Levi et al reported that both basal and stimulated acid secretion plasma gastrin values were significantly higher in H pylori positive patients than in H pylori negative ones. These data were rapidly confirmed, and it was also shown that this increase was reversed after H pylori eradication. Studies of antral biopsies from H pylori infected individuals have shown that gastrin synthesis is increased. Overall, our data are consistent with the notion that the major defect leading to enhanced gastrin concentrations is related to disruption of the inhibitory effect of somatostatin on the G cell.

Normally, gastrin release is suppressed when the luminal antral pH falls below 3. In addition, there is an inhibitory control exerted on gastrin release by cholecystokinin. The inhibition of gastrin release exerted by both gastric acid and cholecystokinin is mediated mainly via the release of somatostatin by D cells within the antral mucosa. Several studies have now demonstrated lowered concentrations of somatostatin within the antral mucosa of subjects with H pylori antral gastritis. In addition, somatostatin mRNA concentrations are lowered, indicating a reduced synthesis of this inhibitory hormone. These findings are consistent with our results that the density of D cells was significantly lower in H pylori infected patients than in uninfected patients and normal individuals, whereas the density of G cells was significantly increased. The mechanism responsible for increased gastrin secretion and reduced somatostatin secretion remains unclear. Hypotheses have included a stimulatory effect of H pylori urease on gastrin release, but this is unlikely because we were unable to show an association between H pylori density and the density of immunoreactive G and D cells. The fact that there was an association with the severity of inflammation is more consistent with a role for cytokines released by inflammatory cells (for example, interleukin 1β, tumour necrosis factor α, and interferon γ). The increase in inflammation associated with the presence of more virulent H pylori types (such as those containing the Cag pathogenicity island) would also be expected to have a more profound effect on gastrin/somatostatin homeostasis.

In some subjects, H pylori infection results in antral predominant gastritis, with increased acid secretion and a propensity to duodenal ulcer disease; in others it results in body gastritis with low acid secretion and a predisposition to atrophic gastritis and gastric cancer, whereas in most subjects it results in mixed gastritis, with no overall change in acid secretion. The net effect on acid secretion presumably depends on which mechanism predominates. Because
patients’ numbers in some subgroups were limited in our study, we could not analyse the group regarding the distribution as antral predominant gastritis, pangastritis, or corpus gastritis.

Overall, our data are consistent with the notion that the major defect leading to enhanced gastrin concentrations is related to disruption of the inhibitory effect of somatostatin on the G cell. It appears that H pylori antral gastritis increases gastrin by producing a deficiency of antral somatostatin and of the normal inhibitory influence of this hormone on gastrin release.

Our study showed that the total numbers of G cells were significantly higher in H pylori infection. However, others have suggested that the numbers are unchanged. The reasons for these differences are unclear. Possibilities include patient selection, as reflected in differences in the pattern and severity of gastritis among those examined. We found that the changes were most pronounced in those with the most severe inflammation. We speculate that the changes in cell numbers may in fact be a dynamic process. Initially, the density of G cells increases, but may later fall if antral atrophy occurs. Subsequent studies are needed to examine the pattern of D and G cells in a wider spectrum of H pylori induced histologically damaged gastric mucosal tissues, and to compare the possible role of different strains of H pylori.

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Gastrin (G) cells and somatostatin (D) cells in patients with dyspeptic symptoms: *Helicobacter pylori* associated and non-associated gastritis

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