

Morphological and functional restoration of parietal cells in *Helicobacter pylori* associated enlarged fold gastritis after eradication

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Abstract

Background/Aim—*Helicobacter pylori* infections are associated with hypochlorhydria in patients with pangastritis. It has previously been shown that eradication of *H pylori* leads to an increase in acid secretion in *H pylori* associated enlarged fold gastritis, suggesting that *H pylori* infection affects parietal cell function in the gastric body. The aim of this study was to evaluate the effects of *H pylori* infection on parietal cell morphology and function in hypochlorhydric patients.

Patients/Methods—The presence of *H pylori* infection, mucosal length, and inflammatory infiltration were investigated in six patients with enlarged fold gastritis and 12 patients without enlarged folds. Parietal cell morphology was examined by immunohistochemistry using an antibody against the α subunit of H^+ , K^+ -ATPase and electron microscopy. In addition, gastric acid secretion and fasting serum gastrin concentration were determined before and after the eradication of *H pylori*.

Results—In the *H pylori* positive patients with enlarged fold gastritis, fold width, foveolar length, and inflammatory infiltration were increased. In addition, the immunostaining pattern of H^+ , K^+ -ATPase was less uniform, and the percentage of altered parietal cells showing dilated canaliculi with vacuole-like structures and few short microvilli was greatly increased compared with that in *H pylori* positive patients without enlarged folds. After eradication, fold width, foveolar length, and inflammatory infiltrates decreased and nearly all parietal cells were restored to normal morphology. On the other hand, altered parietal cells were negligible in *H pylori* negative patients. In addition, the basal acid output and tetragastrin stimulated maximal acid output increased significantly from 0.5 (0.5) to 4.1 (1.5) mmol/h and from 2.5 (1.2) to 13.8 (0.7) mmol/h ($p < 0.01$), and fasting serum gastrin concentrations decreased significantly from 213.5 (31.6) to 70.2 (7.5) pg/ml ($p < 0.01$) after eradication in patients with enlarged fold gastritis.

Conclusion—The morphological changes in parietal cells associated with *H pylori* infection may be functionally associated with the inhibition of acid secretion seen in patients with enlarged fold gastritis.

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Keywords: *Helicobacter pylori*; enlarged fold gastritis; parietal cell morphology; acid secretion; gastrin

Enlarged gastric folds in the gastric body are often observed during radiographic or endoscopic examination of adults. They are associated with a variety of diseases including so called "hypertrophic gastritis", Ménétrier's disease, Zollinger-Ellison syndrome, primary gastrin cell hyperplasia, carcinoma, and lymphoma.^{1,2} Gastric body folds are generally considered to be enlarged when the widest fold is larger than 5 mm based on the Sydney system and previous studies.³⁻⁷ Recently, it has been suggested that *Helicobacter pylori* gastritis may be a possible cause of enlarged gastric folds.^{8,9} *H pylori* associated enlarged fold gastritis⁶ is not an uncommon subgroup of the population with *H pylori* infections. The prevalence of enlarged gastric body folds, as evidenced by a barium study as part of a mass screening for gastric carcinoma, was found to be about 6.3% (68/1079) in Osaka (unpublished data). The prevalence of gastric folds is higher in middle aged (40-59) men (about 11%) than in men in other age groups or in women. We previously reported that eradication of *H pylori* improves fold width in patients with enlarged gastric body folds.⁵

It is now a generally accepted fact that *H pylori* is an important cause of chronic gastritis,¹⁰ peptic ulcer disease,¹¹ and even gastric carcinoma.¹² Under these conditions, acid secretion varies from hypochlorhydria to hyperchlorhydria. *H pylori* infection is also thought to affect gastric secretory functions. It has been previously shown that hypochlorhydria in patients with enlarged gastric body folds is restored to normal levels after eradication of *H pylori*.⁶ However, the morphology of acid secreting parietal cells both before and after eradication of *H pylori* in patients with enlarged gastric body folds has not been fully investigated.

We here report an investigation of the effects of eradicating *H pylori* on the morphology and acid secretion of gastric parietal cells and on the fasting serum gastrin concentration in *H pylori* positive patients with enlarged fold gastritis, and compared these variables with those of *H pylori* positive patients without enlarged folds, as well as *H pylori* negative patients with dyspeptic symptoms.

Abbreviations used in this paper: H^+ , K^+ -ATPase, H^+ and K^+ stimulated adenosine triphosphatase.

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Materials and methods

PATIENTS

The study involved six patients with enlarged fold gastritis (all *H pylori* positive) (four men and two women, mean age 47.5 (3.3) years, range 35–59 years), six *H pylori* positive patients without enlarged folds (three men and three women, mean age 48.3 (3.9) years, range 38–59 years), and six *H pylori* negative patients with dyspeptic symptoms (three men and three women, mean age 45.2 (3.3) years, range 34–56 years). The patients with enlarged fold gastritis had crowded, tortuous, and enlarged folds in the gastric body shown by barium *x* ray performed as part of a mass screen for gastric carcinoma. Subsequently, the patients had an endoscopic examination and were conclusively shown to have inflammation and *H pylori* colonisation of the body mucosa. The patients without enlarged folds had an endoscopic examination to investigate dyspepsia. These patients were divided into two groups on the basis of the presence or absence of *H pylori* infection. A barium *x* ray photograph was taken of each patient by experienced radiologists, using the same equipment and agents. The width of the gastric fold was measured on double contrast radiographs of the appropriately distended stomach with the patient in the supine position. Gastric body folds were considered to be enlarged when the widest fold was more than 5 mm based on previous studies and the Sydney system.^{3–7} None of the patients had peptic ulcer disease or gastric cancer and none were receiving any regular medication and were free of H₂ receptor antagonists, proton pump inhibitors, prostaglandin analogues, and non-steroid anti-inflammatory drugs before and during the period of this study. Five minutes before endoscopy the patients received pharyngeal anaesthesia with lidocaine hydrochloride and an intramuscular injection of butylscopolamine bromide (20 mg). Written informed consent was obtained from each patient before endoscopic examination, and the investigation was approved by the research ethical committee of Osaka University Medical School. Two biopsy specimens were taken from the lesser curvature of the antrum (one for light microscopy and the other for culture studies), and three from the greater curvature of the middle corpus (one for light microscopy, one for culture, and the last for electron microscopic studies). The diagnosis of *H pylori* infection was made based on positive culture results (Department of Chemotherapy, Pharmacological Research Laboratory, Fujisawa Pharmaceutical Co., Osaka, Japan) and/or the result of a urease test (CLO test; Delta West, Bentley, Australia) using biopsy specimens from the antrum and the greater curvature of the upper portion of the gastric body.^{13 14}

MEASUREMENT OF MUCOSAL AND FOVEOLAR LENGTH

The mucosal and foveolar length of the body mucosa were measured on sections vertical to the mucosal surface using an image analysis system (Krypton-40; Flovel). Values were expressed as the mean of measurements along

three or more different foveolae. Glandular length was calculated by subtracting foveolar length from mucosal length.

HISTOLOGICAL EXAMINATION

Biopsy specimens were fixed with 10% phosphate buffered formalin. Thin sections of paraffin wax embedded tissues were stained with haematoxylin and eosin for evaluation of mononuclear and polymorphonuclear infiltration. Infiltrations of mononuclear and polymorphonuclear cells were graded as follows: 0 = none or minimal; 1 = mild; 2 = moderate; and 3 = severe.³ The assessment of gastric atrophy was scored by two pathologists using a number of selected gastric biopsy specimens on a scale of 0 to 3 on the basis of the proportion of glandular loss.^{4 15} Lymphocytic gastritis was diagnosed when the number of intraepithelial lymphocytes was 30% or more of the number of epithelial cells in the areas of high lymphocyte concentration.¹⁶

Immunohistochemical localisation of H⁺ and K⁺ stimulated adenosine triphosphatase (H⁺,K⁺-ATPase) was performed by the peroxidase-antiperoxidase method as previously described¹⁷ using biopsy specimens taken from the greater curvature of the body of the stomach in each patient. A polyclonal rabbit antibody against pig gastric vesicles enriched in H⁺,K⁺-ATPase (provided by Dr M Maeda, Department of Organic Chemistry and Biochemistry, Institute of Scientific and Industrial Research, Osaka University) was used as the primary antibody. It was previously confirmed that this antibody is specific for the 94 kDa H⁺,K⁺-ATPase α subunit by western blot analysis and ELISA.¹⁸ All procedures were performed at room temperature unless otherwise stated. Biopsy samples were fixed in 10% phosphate buffered formalin and embedded in paraffin wax. Paraffin wax sections (about 5 μ m thick) were deparaffinised and dehydrated by passage through xylene and graded concentrations of ethanol. The specimens were then incubated with 3% H₂O₂ solution for 15 minutes to block endogenous peroxidase activity. Sections were then rinsed in distilled water for five minutes and in 0.01 M phosphate buffered saline, pH 7.4, for five minutes. After incubation with normal swine serum diluted 1:20 in phosphate buffered saline for 20 minutes at room temperature, a three step immunoperoxidase procedure was applied, in which, first, the specimens were incubated overnight at 4°C with rabbit antibody against pig gastric vesicles enriched in H⁺,K⁺-ATPase which was diluted 1:1000 in phosphate buffered saline containing 1% bovine serum albumin; secondly, the specimens were incubated for 20 minutes with swine anti-rabbit immunoglobulin (Dakopatts, Copenhagen, Denmark) which was diluted 1:200 in phosphate buffered saline containing 1% bovine serum albumin; thirdly, the specimens were incubated for 20 minutes with peroxidase labelled rabbit anti-peroxidase immunoglobulin complexes (Dakopatts). The reaction was visualised using 5'-diaminobenzidine as chromogen. In addition, the presence or absence of anti-gastric autoantibodies reactive with human gastric mucosa was examined by an

immunohistochemical method described previously.¹⁹

EVALUATION OF ULTRASTRUCTURE

Biopsy specimens for electron microscopy, taken from the greater curvature of the body of the stomach in non-stimulated patients, were immediately fixed in 3% glutaraldehyde solution buffered at pH 7.4 with 0.1 M Millonig's phosphate buffer for two hours at 4°C. Secondary fixation was carried out in 1% osmium tetroxide solution buffered at pH 7.4 with 0.1 M Millonig's phosphate buffer for one hour at 4°C. The specimens were then dehydrated and embedded in epoxy resin (Epon 812). Semithin sections were cut perpendicular to the luminal surface from at least five different tissue blocks for each patient and then stained with 0.5% toluidine blue dissolved in 0.1 M Millonig's phosphate buffer. Ultrathin sections were cut on a Reichert-Jung Ultracut E Ultramicrotome, followed by double staining with 3.0% aqueous uranyl acetate and Reynolds' lead citrate, and were then examined using an Hitachi H-7000 electron microscope. The specimens were analysed semiquantitatively for various features characteristic of abnormal parietal cell ultrastructure. Parietal cells were classified into three groups according to their abnormal ultrastructure.²⁰ Those with dilated canaliculi containing a few short microvilli were classified as altered parietal cells with dilated canaliculi; those with two or more large membrane bound structures containing homogeneous fluffy material and a few small vesicles or membrane remnants were classified as altered parietal cells with vacuoles; those with oedematous cytoplasm and general pallor, which was increased in certain areas as the result of apparent dissolution of the cytosol, were classified as degenerated parietal cells. In such cells, tubulovesicles were partially collapsed and disorganised compared with normal parietal cells. Parietal cells with both dilated canaliculi and vacuoles were often observed and classified as altered parietal cells with vacuoles. For each patient, an average of 400 parietal cells were scored in ultrathin sections from at least five tissue blocks.

DETERMINATION OF BASAL ACID OUTPUT, MAXIMAL ACID OUTPUT, AND SERUM GASTRIN CONCENTRATION

Gastric secretions, basal and stimulated (intravenous injection of 4 µg/kg tetragastrin), were collected for 30 and 60 minutes respectively through a gastric tube with the patient in the left recumbent position, for the determination of basal acid output and maximal acid output. Fasting serum gastrin concentrations were determined by radioimmunoassay (Gastrin RIA Kit II; Dainabot, Tokyo, Japan).²¹

TREATMENT FOR *H PYLORI* INFECTION

Patients with enlarged fold gastritis (all of which were *H pylori* positive) received triple agent treatment with bismuth subnitrate or bismuth subcarboxylate 1 g three times a day, 250 mg of metronidazole twice a day, and tetracycline hydrochloride 250 mg four times a day for three weeks.⁶⁻¹⁰ At 4–23 weeks after the completion of this treatment schedule, the

endoscopic examinations, fasting serum gastrin determinations, and gastric secretion measurements were repeated. At 7–25 weeks after the completion of the treatment, the upper gastrointestinal barium x ray examination was repeated for reassessment of the size of the folds. Eradication of *H pylori* was successful in all patients.

STATISTICAL ANALYSIS

Data are expressed as mean (SEM). Statistical analyses were performed using the Wilcoxon signed rank test and the Mann-Whitney test. Pearson's correlation coefficient was also determined. $p < 0.05$ was considered statistically significant.

Results

H PYLORI STATUS

For all six patients with enlarged fold gastritis and in six of the 12 patients without enlarged folds, *H pylori* was present in both the antrum and the body except in one patient with enlarged fold gastritis who was positive for *H pylori* only in the gastric body.

MUCOSAL LENGTH

The gastric body fold width in patients with enlarged fold gastritis was significantly reduced from 9.7 (0.8) to 6.0 (0.4) mm after eradication ($p < 0.01$) (table 1). The fold width in the *H pylori* positive patients without enlarged folds was significantly greater than that in the *H pylori* negative patients ($p < 0.01$). In patients with enlarged fold gastritis, the mucosal and foveolar length of the body mucosa were significantly greater than those in both the *H pylori* positive and negative patients without enlarged folds ($p < 0.01$). The foveolar/glandular length ratio in the patients with enlarged fold gastritis was significantly higher than that in the *H pylori* negative patients without enlarged folds ($p < 0.01$) (table 1). No significant differences in mucosal, foveolar, and glandular length, and foveolar/glandular length ratio between the *H pylori* positive and negative patients without enlarged folds was observed.

INFLAMMATORY CELL INFILTRATION AND MUCOSAL ATROPHY

Inflammatory cell infiltration was mainly confined to the foveolar and surface epithelial layer. A histological examination showed that mononuclear and polymorphonuclear infiltrates in the body mucosa, as well as in the antral mucosa, of the patients with enlarged fold gastritis were significantly more severe than those in the *H pylori* negative patients without enlarged folds ($p < 0.01$). Inflammatory cell infiltration in the body mucosa in the patients with enlarged fold gastritis was more severe than that in *H pylori* positive patients without enlarged folds, but this difference was not statistically significant. The *H pylori* positive patients without enlarged folds showed appreciably more extensive infiltration of inflammatory cells than did the *H pylori* negative patients (table 2). Neither mononuclear nor polymorphonuclear cell infiltrates were observed in *H pylori* negative patients. No typical features of lymphocytic gastritis were observed in any of the patients.

Table 1 Fold width, mucosal, foveolar, glandular length, and foveolar:glandular length ratio of the body mucosa

	Fold width (mm)	Mucosal length (μ m)	Foveolar length (μ m)	Glandular length (μ m)	Foveolar: glandular length ratio
Enlarged fold gastritis (n = 6)					
Before eradication	9.7 (0.8) (7–12)*†	1014 (44)*†	472 (20)*†	543 (2)6‡	0.87 (0.02)*
After eradication	6.0 (0.4) (7–12)*‡	805 (60)**†‡	284 (35)**†‡	525 (42)	0.56 (0.08)‡
Non-enlarged fold (n = 12)					
<i>H pylori</i> positive (n = 6)	4.5 (0.2) (4–5)*	606 (20)	182 (7)	424 (18)	0.43 (0.02)
<i>H pylori</i> negative (n = 6)	2.5 (0.2) (2–3)	586 (34)	160 (18)	425 (32)	0.39 (0.06)

Results are expressed as mean (SEM) (range).

* $p < 0.01$ and ** $p < 0.05$ v non-enlarged fold, *H pylori* negative.

† $p < 0.01$ and †† $p < 0.05$ v non-enlarged fold, *H pylori* positive.

‡ $p < 0.01$ and ‡‡ $p < 0.05$ v before.

Table 2 Inflammatory infiltrates in the body and antral mucosa

	Mononuclear cell infiltrates (grade no)		Polymorphonuclear cell infiltrates (grade no)	
	Body	Antrum	Body	Antrum
Enlarged fold gastritis (n = 6)				
Before eradication	1.8 (0.3)*	2.2 (0.3)*	1.3 (0.2)*	1.0 (0.4)*
After eradication	1.0 (0.0)**†‡	1.0 (0.3)†‡	0.0 (0.0)†‡	0.2 (0.2)†‡
Non-enlarged fold (n = 12)				
<i>H pylori</i> positive (n = 6)	1.7 (0.2)*	1.8 (0.2)*	0.8 (0.3)**	1.0 (0.3)*
<i>H pylori</i> negative (n = 6)	0.5 (0.2)	0.5 (0.2)	0.0 (0.0)	0.2 (0.2)

All results are expressed as mean (SEM).

Enlarged fold gastritis is gastritis accompanied by enlarged folds (the widest fold > 5 mm).

Four grades; 0 = none or minimal; 1 = mild; 2 = moderate; 3 = severe.

* $p < 0.01$ and ** $p < 0.05$ v non-enlarged fold, *H pylori* negative.

† $p < 0.05$ v non-enlarged fold, *H pylori* positive.

‡ $p < 0.01$ and ‡‡ $p < 0.05$ v before.

No significant difference in gastric body atrophy score was found among the six patients with enlarged fold gastritis, the six *H pylori* positive patients without enlarged folds, or the six *H pylori* negative patients (0.3 (0.2), 0.3 (0.2), and 0.0 respectively). After eradication, there was no significant change in the degree of body atrophy in patients with enlarged fold gastritis.

IMMUNOSTAINING FOR H^+ , K^+ -ATPase IN Parietal Cells

Immunostaining specific for the α subunit of H^+ , K^+ -ATPase was specifically localised to the parietal cells. However, different staining patterns were observed among patients with enlarged fold gastritis. In the *H pylori* positive patients without enlarged folds and *H pylori* negative patients, the immunostaining pattern for H^+ , K^+ -ATPase was uniform throughout the cytoplasm (fig 1A), whereas cytoplasmic H^+ , K^+ -ATPase staining in parietal cells was less uniform in patients with enlarged fold gastritis. In some parietal cells, positively stained cytoplasm was interrupted by several clear H^+ , K^+ -ATPase negative vacuole-like areas (fig 1B). After *H pylori* eradication, the vacuole or vacuole-like structures and dilated canaliculi had nearly disappeared. The staining pattern of the H^+ , K^+ -ATPase became homogeneous in the cytoplasm of parietal cells and similar to that in tissues from *H pylori* negative patients. Also, no anti-gastric autoantibody could be detected in sera of patients with enlarged fold gastritis.

LIGHT MICROSCOPY OF GASTRIC MUCOSA SEMITHIN SECTIONS

To examine the morphological differences in parietal cells between the patients with enlarged fold gastritis and those without enlarged folds

(the *H pylori* negative and *H pylori* positive), semithin sections of Epon embedded gastric mucosal tissue stained with toluidine blue were examined by light microscopy. In the tissue from *H pylori* negative patients, normal parietal cells appeared as large pyramidal or spherical cells with a central nucleus, and the cytoplasm was dotted with mitochondria (fig 2A). On the other hand, many parietal cells in the *H pylori* positive patients with enlarged folds showed morphological changes (fig 2B). They often contained abnormally dilated canaliculi or vacuole-like structures, which appeared to be different from canaliculi. After *H pylori* eradication, the appearance of most parietal cells returned to normal. Altered parietal cell morphology was only rarely observed in *H pylori* positive patients without enlarged folds.

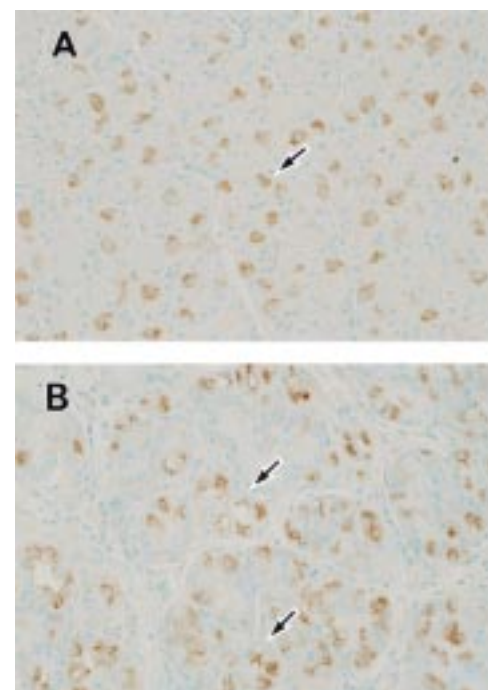


Figure 1 Immunohistochemical detection of H^+ , K^+ -ATPase in human gastric mucosa. (A) Gastric mucosal tissue of an *H pylori* negative patient without enlarged folds. Parietal cell cytoplasm is specifically and uniformly stained (arrow). Original magnification $\times 210$. (B) Gastric mucosal tissue of an *H pylori* positive patient with enlarged fold gastritis. Some parietal cells are uniformly stained as above. In other parietal cells, stained cytoplasm is less uniform and appears granular or contains several vacuole-like clear areas which are negatively stained for H^+ , K^+ -ATPase (arrows). Original magnification $\times 210$.

ELECTRON MICROSCOPY OF PARIETAL CELLS

Electron microscopy showed that *H pylori* was present in the mucous gel layer but was often located close to the surface mucous cells in patients with enlarged fold gastritis (fig 3).

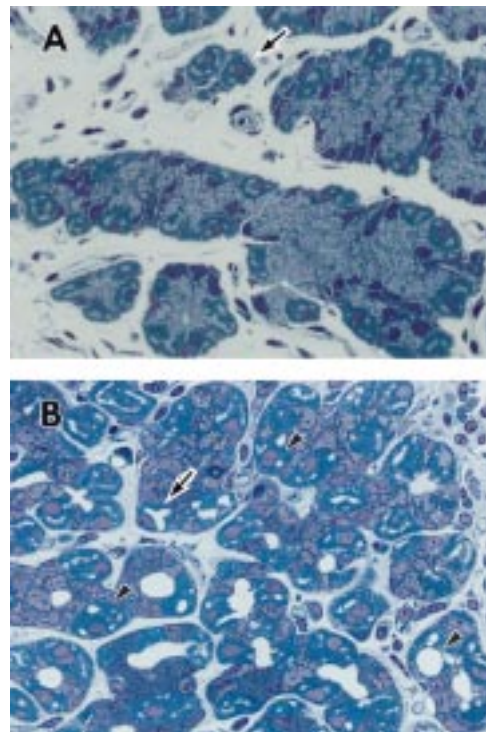


Figure 2 Semithin plastic sections of gastric mucosa stained with toluidine blue. (A) Gastric mucosal tissue from an *H pylori* negative patient without enlarged folds. Parietal cells appear large and pyramidal or spherical with central nuclei (arrow). Original magnification $\times 210$. (B) Gastric mucosal tissue from an *H pylori* positive patient with enlarged fold gastritis. Extensive infiltration of inflammatory cells in the lamina propria is visible. Numerous parietal cells show abnormally dilated canaliculi (arrow) and sometimes vacuole-like structures which are different from canaliculi (arrowhead). Original magnification $\times 210$.

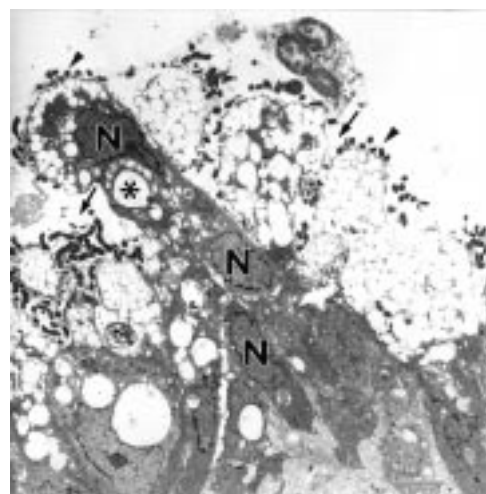


Figure 3 Low magnification electron micrograph of the gastric mucosal lumen in an *H pylori* positive patient with enlarged fold gastritis. N, nucleus. Numerous bacteria (arrowheads) are observed in the mucous layer and close to gastric epithelial cells. There is considerable superficial vacuolation (*), evidence of loss of microvilli, and depletion of mucin granules. The organisms are also observed in the foveolar and intercellular spaces (arrows). Original magnification $\times 2000$ (bar = $5 \mu\text{m}$).

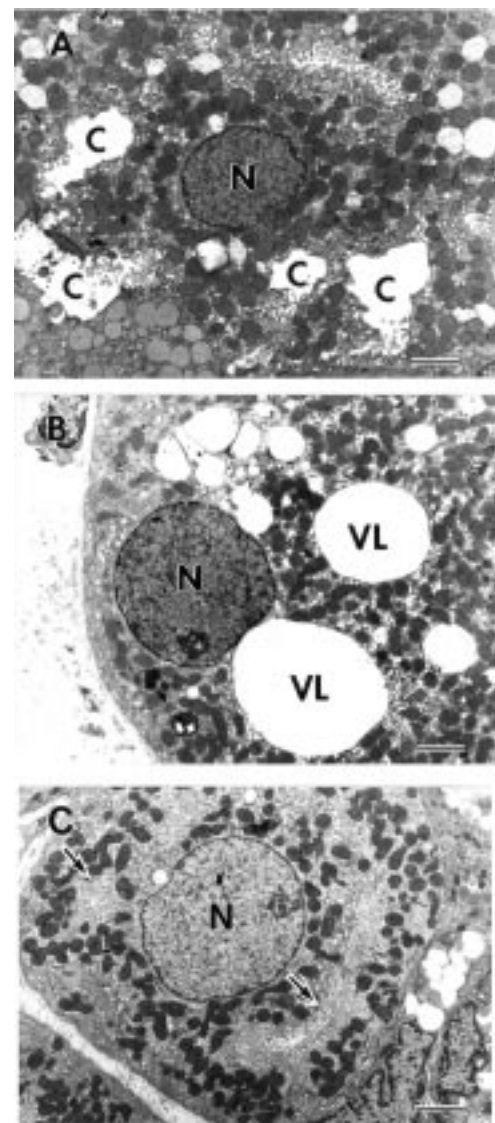


Figure 4 Electron micrographs of parietal cells in an *H pylori* positive patient with enlarged fold gastritis before (A, B) and after (C) *H pylori* eradication. N, nucleus; C, intracellular canaliculus. (A) This parietal cell is classified as an altered parietal cell with dilated canaliculi. It has dilated canaliculi containing few short microvilli (bar = $3 \mu\text{m}$). (B) This parietal cell is classified as an altered parietal cell with vacuoles. Three large spherical electron lucent structures interpreted to be a vacuole-like structure (VL) are present in the cytoplasm, and microvilli at the luminal surface have completely disappeared (bar = $3 \mu\text{m}$). (C) After eradication of *H pylori* in patients with enlarged fold gastritis, the parietal cell has almost reverted to a normal appearance as seen in *H pylori* negative patients. Most of the cytoplasm is occupied by tubulovesicles and mitochondria, and canaliculi with developed microvilli can be recognised (arrows) (bar = $3 \mu\text{m}$). Original magnification: A, $\times 4000$; B, $\times 4000$; C, $\times 4000$.

Parietal cell ultrastructure was examined in all patients. Parietal cells showed no signs of poor tissue preservation such as swelling of mitochondria and dilatation of nuclear membrane. Most parietal cells in the gastric mucosal tissue of the *H pylori* negative patients were in the typical resting state, with small canaliculi, short microvilli, and numerous tubulovesicles (data not shown). In contrast, numerous parietal cells in *H pylori* positive patients with enlarged fold gastritis showed atypical ultrastructures. These parietal cells often exhibited abnormally dilated canaliculi containing few short micro-

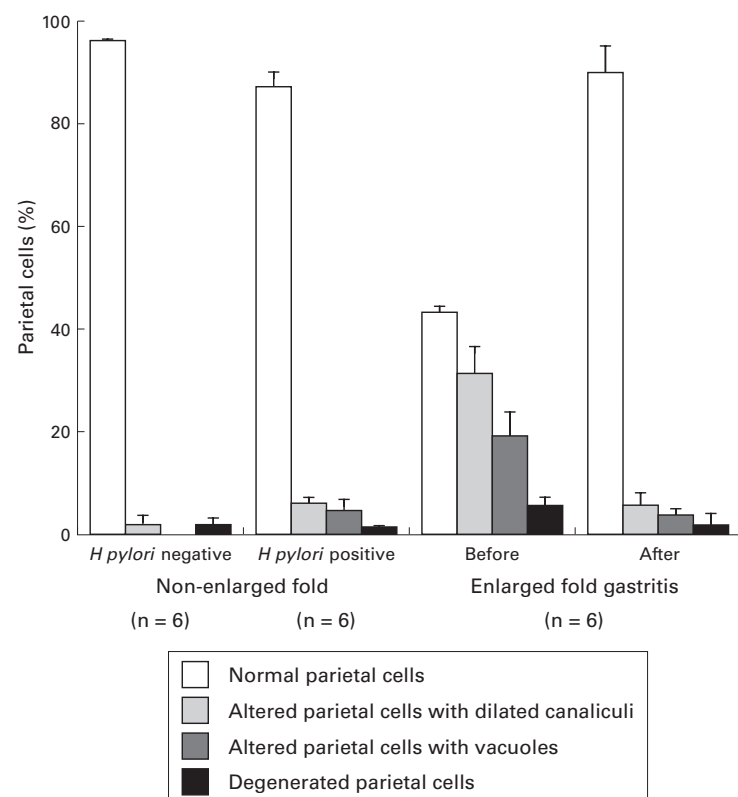


Figure 5 Semiquantitative analysis of parietal cell morphology before and after eradication of *H. pylori* in six patients with enlarged fold gastritis and 12 patients without enlarged folds (six *H. pylori* positive and six *H. pylori* negative). Electron micrographs of gastric mucosa were scored according to parietal cell morphology as being normal, altered with dilated canaliculi, altered with vacuoles, or degenerated. The mean of the percentage of each class is represented by bars. Standard errors are represented by vertical lines.

villi (fig 4A), and sometimes spherical light cytoplasmic areas resembling vacuole-like structures were displayed (fig 4B). Some parietal cells appeared pale, probably because of early necrosis. Macrophage infiltration of the lamina propria was greater in *H. pylori* positive patients with enlarged fold gastritis (data not shown). After *H. pylori* eradication in these patients, normal parietal cells with a resting morphology predominated (fig 4C).

Parietal cells were scored for abnormal ultrastructure as normal, altered, or degenerated on the basis of the criteria described in Materials and methods; about 400 parietal cells per patient were scored from the electron micrographs. In the *H. pylori* negative patients, 3.5 (0.2)% of parietal cells were altered, consistent with previously reported data in mice.²² Altered or degenerated parietal cells were rare at the isthmus and neck, and 6.7 (0.4)% of parietal cells in the base of the gastric gland were altered or degenerated.

It is noteworthy that, in the *H. pylori* positive patients with enlarged fold gastritis, 56.6 (1.2)% of the parietal cells were altered. Of these altered cells, 31.5 (4.2)% contained dilated canaliculi, 19.5 (3.7)% contained vacuole-like structures, and 5.6 (1.4)% were degenerated. Altered and degenerated parietal cells were consistently encountered at the isthmus, neck, and base of the gastric gland. Thus the percentage of altered and degenerated parietal cells was greatly increased in *H. pylori*

positive patients with enlarged fold gastritis compared with that in both *H. pylori* positive patients without enlarged folds and *H. pylori* negative patients. After *H. pylori* eradication in *H. pylori* positive patients with enlarged fold gastritis, parietal cell ultrastructure was generally similar to that in *H. pylori* negative patients (fig 4C). Consequently, the percentage of normal parietal cells significantly increased to 90.2 (2.3), and the percentage of parietal cells with dilated canaliculi, vacuole-like structures, and degeneration significantly decreased to 5.8 (1.2), 3.8 (0.6) and 1.6 (0.9) respectively (fig 5).

BASAL ACID OUTPUT, MAXIMAL ACID OUTPUT, AND SERUM GASTRIN CONCENTRATION

Figure 6 shows basal acid output, tetragastrin stimulated maximal acid output, and serum gastrin concentrations before and after eradication of *H. pylori* in the patients with enlarged fold gastritis (all *H. pylori* positive), the *H. pylori* positive patients without enlarged folds, and the *H. pylori* negative patients. Basal acid output in the patients with enlarged fold gastritis before *H. pylori* eradication was much lower than in both the *H. pylori* positive patients without enlarged folds and *H. pylori* negative patients (0.5 (0.5) v 3.5 (0.2) v 5.0 (0.1) mmol/h respectively; $p < 0.01$) (fig 6A). Likewise, tetragastrin stimulated maximal acid output in patients with enlarged fold gastritis before *H. pylori* eradication was much lower than in both the *H. pylori* positive patients without enlarged folds and *H. pylori* negative patients (2.5 (1.2) v 11.7 (1.5) v 15.5 (1.4) mmol/h respectively; $p < 0.01$) (fig 6B). After *H. pylori* eradication, basal acid output and tetragastrin stimulated maximal acid output significantly increased in patients with enlarged fold gastritis to 4.1 (1.5) mmol/h and to 13.8 (0.7) mmol/h ($p < 0.01$) respectively. Serum gastrin concentration in the patients with enlarged fold gastritis was much higher than in both the *H. pylori* positive patients without enlarged folds and *H. pylori* negative patients (213.5 (31.5) v 77.5 (10.9) v 50.7 (7.6) pg/ml respectively; $p < 0.01$) (fig 6C). After eradication of *H. pylori* in patients with enlarged fold gastritis, the serum gastrin concentration significantly decreased to 70.2 (7.5) pg/ml ($p < 0.01$).

Discussion

This study clearly shows that gastric body fold width is decreased and that an associated improvement in mucosal inflammatory infiltrates, especially polymorphonuclear infiltrates, was achieved as the result of the eradication of *H. pylori* in *H. pylori* positive patients with enlarged fold gastritis. Consistent with our findings, the return to normal of giant folds after *H. pylori* eradication in the patients with Ménétrier's diseases has been reported by Bayerdörffer *et al.*²³ Ménétrier's disease, which is histologically characterised as a form of hypertrophic gastropathy, involves massive foveolar hyperplasia, little, if any, inflammation, and cystic dilatations of the body mucosa. These criteria have been reported by Wolfsen *et al.*²⁴ who showed that the medians (range) for

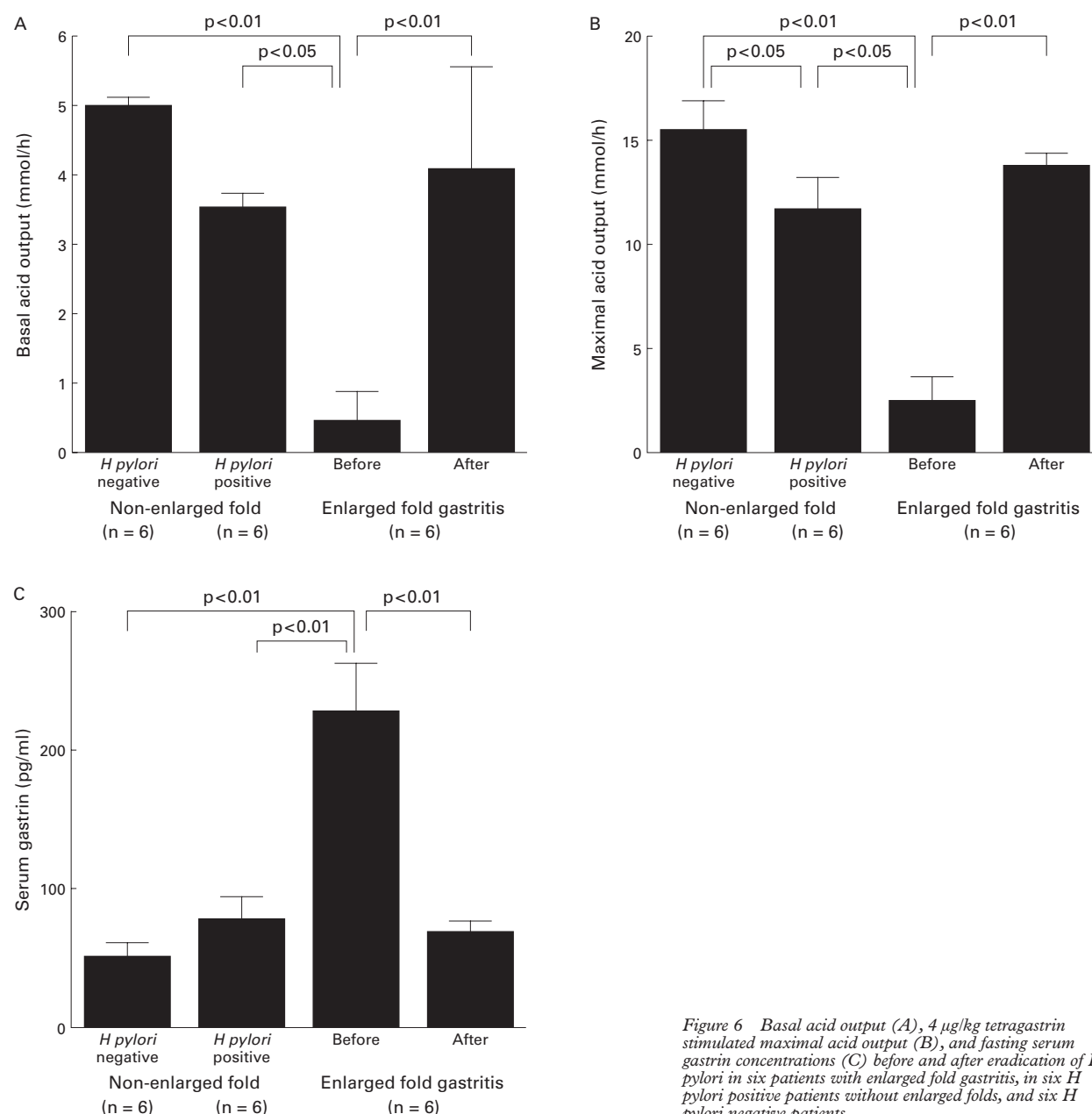


Figure 6 Basal acid output (A), 4 µg/kg tetragastrin stimulated maximal acid output (B), and fasting serum gastrin concentrations (C) before and after eradication of *H. pylori* in six patients with enlarged fold gastritis, in six *H. pylori* positive patients without enlarged folds, and six *H. pylori* negative patients.

mucosal thickness and the foveolar/gland ratio in patients with classic Ménétrier's disease are 5.5 (2.5–12.5) mm and 9.5 (3–15) respectively. In our study, the medians (range) for mucosal length and the foveolar/gland ratio in patients with enlarged fold gastritis are 1.014 (0.876–1.167) mm and 0.87 (0.80–0.94) respectively. Therefore the histological findings for patients with enlarged folds in our study can be considered to be distinguishable from classic Ménétrier's disease.²⁴ In addition, this study shows that mucosal length, especially foveolar length, of the body mucosa is increased in patients with enlarged fold gastritis. These results suggest that thickening of the gastric folds in enlarged fold gastritis is due to the combination of foveolar hyperplasia and inflammatory infiltrates in the lamina propria.

In this study we investigated the gastric mucosal morphology and function, with em-

phasis on the ultrastructural features of parietal cells, in patients with enlarged fold gastritis associated with *H. pylori* infection. Parietal cells in these patients showed a variety of abnormal features such as considerably dilated canaliculi containing few short microvilli, round electron lucent structures resembling vacuole-like structures, degeneration, and the presence of *H. pylori* organelles. These findings are consistent with a previous report by Taguchi *et al.*²⁵ The percentage of abnormal parietal cells in *H. pylori* positive patients with enlarged fold gastritis was higher than in *H. pylori* positive patients without enlarged folds and *H. pylori* negative patients. This study also shows that, after eradication of *H. pylori* in patients with enlarged fold gastritis, parietal cell morphology and gastric acid secretion returned to normal.

Parietal cell morphology is different in the resting and stimulated states.²⁶ In the resting

state, parietal cells are characterised by small secretory canaliculi with few short microvilli and numerous tubulovesicles in the cytoplasm. In the stimulated state, a considerable increase in dilated secretory canaliculi with more and longer microvilli with a concomitant decrease in the number of tubulovesicles in the cytoplasm is observed. Parietal cell morphology in *H pylori* positive patients with enlarged fold gastritis is distinctly different from that of resting or stimulated parietal cells. In addition, basal and maximal acid outputs are significantly decreased in *H pylori* positive patients with enlarged fold gastritis before eradication. The ultrastructural changes in parietal cells in these patients is thought to reflect the functional status of these cells.²⁷⁻³¹ It thus appears that the changes in parietal cell morphology, which concomitantly occur with *H pylori* infection, and the increase in altered parietal cells are associated with decreased acid secretion in *H pylori* positive patients with enlarged fold gastritis. It follows therefore that altered parietal cells do not function normally.

Semiquantitative ultrastructural analysis showed parietal cell morphologies in the gastric glands in *H pylori* positive patients with enlarged fold gastritis that were rarely observed in *H pylori* negative patients. Moreover, more altered parietal cells were present in all the glands of every patient with *H pylori* positive enlarged fold gastritis than in *H pylori* negative patients. At 4-23 weeks after *H pylori* eradication, the percentage of altered parietal cells had decreased significantly and the percentage of normal parietal cells had reached nearly the same level as in *H pylori* negative patients. Thus the percentage of altered parietal cells returned to normal levels in a relatively short period of time. Also, basal acid output and tetragastrin stimulated maximal acid output recovered to normal acid levels, and no significant change in the degree of the body atrophy was observed after *H pylori* eradication. The normal turnover time of parietal cells is relatively long, about 54 days in the mouse,²² 200 (100) days in the golden hamster,³² and one to several years in human.³³ It has been reported that inhibition of gastric acid secretion does not significantly affect the turnover rate of parietal cells.³⁴ Based on the literature and the results herein, the altered parietal cells that contain vacuole-like structures may not be replaced parietal cells, but pre-existing ones that have functionally changed.

This study shows that the H⁺, K⁺-ATPase staining in parietal cells was less uniform in patients with enlarged fold gastritis. After *H pylori* eradication, the staining pattern of the H⁺, K⁺-ATPase became homogeneous in the cytoplasm of parietal cells, and was similar to that in tissues from *H pylori* negative patients. As the presence of anti-gastric autoantibodies due to *H pylori* infection may affect the morphological and/or functional changes in the parietal cells, as suggested by Faller *et al*,¹⁹ the presence or absence of anti-gastric autoantibody in our patients with enlarged fold gastritis was investigated by immunohistochemistry. The data show that no anti-gastric autoantibody was present in the sera of these patients

with enlarged fold gastritis, as evidenced by our method. Therefore it is unlikely that the H⁺, K⁺-ATPase staining pattern in patients with enlarged fold gastritis before *H pylori* eradication is due to anti-gastric autoantibodies.

The exact mechanism by which *H pylori* infection induces decreased gastric acid secretion and alterations in parietal cell morphology remains unclear. Four possible mechanisms may be involved. First, parietal cell function may be directly affected by *H pylori*. It has been reported that *H pylori* is occasionally observed in the canaliculi of human parietal cells.³⁵ However, this mechanism seems unlikely as *H pylori* was rarely observed in parietal cells in our study. Second, changes in the number of parietal cells per stomach after eradication of *H pylori* may cause an increase in acid secretion. However, there was no appreciable change in the degree of body atrophy before and after *H pylori* eradication. The lifespan of parietal cells is known to be relatively long.^{22 32 33} In a relatively short period, the morphology and acid output of parietal cells returned to normal in our study. It is therefore unlikely that this mechanism is a major contributor to the increase in acid secretion. Third, an acid inhibitory protein composed of two 46 kDa subunits^{36 37} or fatty acids³⁸ that are synthesised by *H pylori* may have an effect on parietal cell function. Fourth, the cytokines produced by local immune system cells during inflammation³⁹ may indirectly affect gastric function. In this study, it was observed that the inflammatory cell infiltration in the gastric body mucosa was much more extensive in the *H pylori* positive patients with enlarged fold gastritis. In a previous study, it was observed that fundic mucosal interleukin 1 β and hepatocyte growth factor production were enhanced in *H pylori* associated enlarged fold gastritis. It was suggested that interleukin 1 β was involved in the inhibition of acid secretion, and that hepatocyte growth factor was involved in fold enlargement through stimulation of epithelial cell proliferation and foveolae dominant mucosal hyperplasia.⁷ Interleukin 1 β in particular is a potent inhibitor of gastric acid secretion in conscious animals.^{40 41} Therefore *H pylori* infection may reduce acid secretion indirectly by inducing an immune response which leads to production of interleukin 1 β . Further study is clearly necessary to elucidate the specific mechanisms responsible for hypochlorhydria and altered parietal cell morphology.

This study further shows that the percentage of altered parietal cells in the *H pylori* positive patients with enlarged fold gastritis was higher than in the *H pylori* positive patients without enlarged folds despite the fact that both groups of patients were *H pylori* positive. This may be due to differences in the abundance of *H pylori* organisms in the oxyntic mucosa and to the extent of damage and inflammation. It has been reported that omeprazole, which is a powerful inhibitor of gastric acid production, causes increased colonisation of *H pylori* in the fundus.⁴² Thus, in the *H pylori* positive patients with enlarged fold gastritis, the evolving inflammation in the oxyntic mucosa may lead

to a gradual decrease in acid secretion, which may, in turn, create favourable conditions for gradual *H. pylori* colonisation of the oxyntic mucosa, initiating a vicious cycle of increasing *H. pylori* colonisation of the oxyntic mucosa and decreasing acid secretion.

In conclusion, this study has shown morphological changes in parietal cells during *H. pylori* infection for the first time, and demonstrated that these changes are associated with decreased acid secretion in *H. pylori* positive patients with enlarged gastric body fold gastritis.

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Morphological and functional restoration of parietal cells in *Helicobacter pylori* associated enlarged fold gastritis after eradication

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