

Helicobacter pylori related hypergastrinaemia is the result of a selective increase in gastrin 17

G Mulholland, J E S Ardill, D Fillmore, R S Chittajallu, G M Fullarton, K E L McColl

Abstract

Helicobacter pylori infection increases the serum concentration of gastrin, and this may be one of the mechanisms by which it predisposes to duodenal ulceration. Different forms of circulating gastrin were studied both basally and postprandially in 13 duodenal ulcer patients before and one month after eradication of *H pylori*. Three antisera that are specific for particular regions of the gastrin molecules were used. Gel chromatography indicated that >90% of the circulating gastrin consisted of gastrin (G) 17 and G34 both before and after eradicating the infection. The basal median total immunoreactive gastrin concentration fell from 26 pmol/l (range 11-43) to 19 pmol/l (8-39) ($p < 0.05$), entirely because of a fall in G17 from 6 pmol/l (<2.4-25) to <2.4 pmol/l (<2.4-23) ($p < 0.001$). The median (range) basal G34 values were similar before (15 pmol (2-36)) and after (10 pmol (2-30)) eradication. The median total immunoreactive gastrin concentration determined 20 minutes postprandially fell from 59 pmol/l (38-114) to 33 pmol/l (19-88) ($p < 0.005$), and again this was entirely the result of a fall in G17 from 43 pmol/l (9-95) to 17 pmol/l (<2.4-52) ($p < 0.001$). The median postprandial G34 values were similar before (13 pmol/l, range 6-42) and after (15 pmol/l, range 6-30) eradication. Eating stimulated a noticeable rise in G17 but little change in G34, both in the presence and absence of *H pylori*. The finding that *H pylori* infection selectively increases G17 explains why the infection causes mainly postprandial hypergastrinaemia. G17 is increased selectively because *H pylori* predominantly affects the antral mucosa which is the main source of G17 whereas G34 is mainly duodenal in origin. This study also indicates that the increased concentration of gastrin in *H pylori* infection is the result of an increase in one of the main biologically active forms of the hormone.

(Gut 1993; 34: 757-761)

Numerous studies have shown that *Helicobacter pylori* infection of the gastric antrum raises circulating gastrin concentrations in duodenal ulcer patients and healthy volunteers.¹⁻⁶ The basal concentrations are increased by approximately 50%, and the integrated gastrin response to a meal is increased by approximately 100%. The mechanism by which *H pylori* infection increases serum gastrin is unknown but does not seem to be related to the bacterium's urease activity⁷⁻⁹ or any effect of this on antral surface pH.¹⁰

The role of the increased serum gastrin concentration induced by *H pylori* in the pathogene-

sis of duodenal ulcer disease is also unknown. Studies to date have produced conflicting evidence concerning changes in acid secretion after eradication of *H pylori* and lowering of the serum gastrin concentration.^{2,3,11,12} The reason for this is unclear. One possible explanation is that the increased immunoreactive gastrin circulating in patients with *H pylori* infection is of reduced biological activity or indeed not biologically active at all.

It is established that gastrin circulates in at least four bioactive forms, component I,¹³ gastrin (G) 34,¹⁴ G17,¹⁵ and G14.¹⁶ It has been suggested by Akai *et al*¹⁷ that some gastrin may be produced by an alternative processing route, which may involve other intermediate forms. Kothary *et al*¹⁸ have reported that terminally extended forms of gastrin in conjunction with G14 are more prevalent in duodenal ulcer patients. Other fragments including N-terminal fragments of G34 have also been identified.¹⁹ It is possible that the inflammation of the antral mucosa induced by *H pylori* infection disturbs the intracellular processing of gastrin by the antral G cells.

There have been conflicting reports concerning the form of gastrin that is increased in *H pylori* infection. Beardshall *et al*²⁰ have reported that the increased gastrin response to gastrin releasing peptide in *H pylori* infection is the result of increased G17. Graham *et al*,²¹ however, have claimed that the exaggerated meal stimulated gastrin response caused by *H pylori* is the result of an increase in biologically inactive progastrin. To clarify this, we have examined the different circulating forms of gastrin in duodenal ulcer patients, basally and in response to eating, and both before and after eradication of *H pylori*.

Subjects and methods

PATIENTS

Thirteen patients (three women, age range 25-64 years) with a history of duodenal ulceration were studied. *H pylori* infection was confirmed in each by microscopy of endoscopic antral biopsy tissue, urease slide test (CLO-test) of antral biopsy tissue, and positive ¹⁴C-urea breath test. All patients stopped taking any acid inhibitory agents at least two weeks before entering the study and none had received bismuth preparations in the past.

METHODS

The patients reported at 0900 h after an overnight fast. Three venous blood samples (10 ml each) were taken over 30 minutes for fasting gastrin estimation. The patients then drank over 5 minutes a peptone meal consisting of two beef

University Department of
Medicine and
Therapeutics, Western
Infirmary, Glasgow
G11 6NT
R S Chittajallu
G M Fullarton
K E L McColl

Department of Medicine,
Queen's University,
Belfast

G Mulholland
J E S Ardill
D Fillmore

Correspondence to:
Dr K E L McColl.

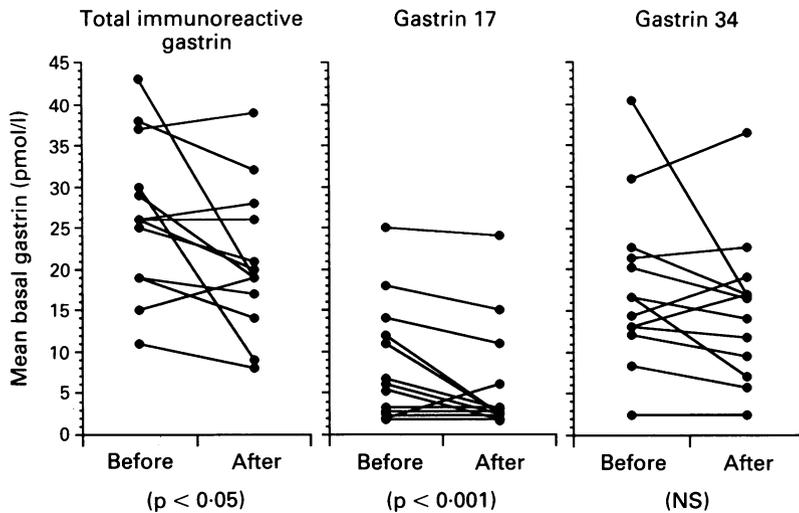


Figure 1: Fasting serum concentrations of total immunoreactive gastrin (IRG) (R98), gastrin 17 (GP168), and gastrin 34 (subtraction assay) before and one month after eradication of *Helicobacter pylori* infection.

cubes (OXO Ltd, Croydon, England) dissolved in 200 ml water at 60°C. A blood sample was taken five minutes after completing the drink and further samples were taken at 10 minute intervals for 90 minutes.

Patients then received a three week course of tripotassium dicitrato-bismuthate (De-Nol) (120 mg four times daily), metronidazole (400 mg three times daily), and amoxicillin (500 mg three times daily). One month after completing this treatment, the ¹⁴C-urea breath test was repeated to assess eradication of the infection. At this time the basal and meal stimulated gastrin concentrations were reassessed as described above.

ANALYSIS

The venous blood samples were allowed to clot over 10 minutes and the serum separated and stored at -20°C. Preliminary studies were undertaken in which random blood samples were obtained from 15 subjects and processed as serum, EDTA plasma, and lithium heparin plasma. These showed no statistical difference in

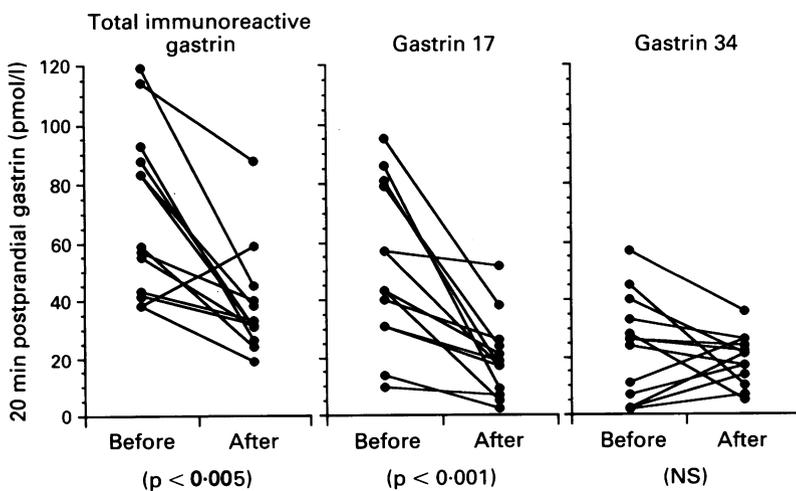


Figure 2: Meal stimulated serum concentrations of total immunoreactive gastrin (IRG) (R98), gastrin 17 (GP168), and gastrin 34 (subtraction assay) before and one month after eradication of *Helicobacter pylori*.

the concentrations of the various forms of gastrins between the different methods of sample preparation.

The gastrin measurements were performed by standard radioimmunoassay techniques using three antisera, R98, GP168, and R526.

Antisera R98 was raised in rabbit to synthetic un sulphated human gastrin 2-17 coupled to chicken egg albumen using glutaraldehyde.²² It binds component I, G34, G17, and G14 in sulphated and un sulphated forms with equimolar potency. Cross reaction with cholecystokinn (CCK) is <0.00005. The coefficient of interassay variation is 3.8% at 10 pmol/l, 3.3% at 20 pmol/l, and 5.4% at 40 pmol/l.

Antisera GP168 was raised in guinea pig to synthetic human gastrin fragment 1-13 of G17 coupled to chicken egg albumen using carbodiimide. It binds G17 in the sulphated and un sulphated forms with equimolar potency and also fragments 1-6 and 1-13 of G17. It does not bind component I, G34, or G14 and shows no cross reaction with CCK. The coefficient of interassay variation is 10.1% at 10 pmol/l, 7.3% at 20 pmol/l, and 3.8% at 40 pmol/l.

Antisera R526 was raised in rabbit to synthetic human gastrin fragment 1-17 of G34, coupled to chicken egg albumen using glutaraldehyde. It binds G34 in the sulphated and un sulphated forms and also binds N-terminal fragments of G34. It does not bind G17 and G14. The coefficient of interassay variation is 12.1% at 10 pmol/l, 8.5% at 20 pmol/l, and 6.2% at 40 pmol/l.

The tracer used with both R98 and GP168 antisera was mono-iodinated (Leu15) human G17. The detection limit of both assays was <2.4 pmol/l as used routinely. The tracer used with R526 was mono-iodinated human G34. The detection limits of the assay was 5 pmol. All specimens were assayed in at least two dilutions and samples taken before and after eradication were assayed within the same batch.

G34 was assayed using antisera R526 in only eight patients in order to preserve sufficient sample for chromatographic studies. G34 was estimated using subtraction of G17 (GP168) from total gastrins (R98) in all 13 patients. The interassay variation for the subtraction assay is 10.4% at 20 pmol/l, as determined by repeated measurements of clinical samples.

Chromatographic analysis was performed on serum samples obtained 20 minutes postprandially before and after eradication of *H pylori* in six patients. This was undertaken to determine the contribution of G17 and G34 to the total immunoreactive gastrin in the presence and absence of *H pylori*. The 2.5 ml samples were applied to a G50 superfine column (0.9×100 cm). The samples were eluted using phosphate buffer 0.1 M pH 7.2 that contained human albumen 0.1 g% (RIA grade). The flow rate was 4 ml/hour. All eluates were assayed with the three assays described above. Recovery of gastrins from the column was >80%.

The mean value of the fasting samples obtained at 15 minutes and immediately before the meal was taken as the basal gastrin value. Statistical analysis was performed by means of the Wilcoxon rank sum test for paired data.

The study was approved by the Western

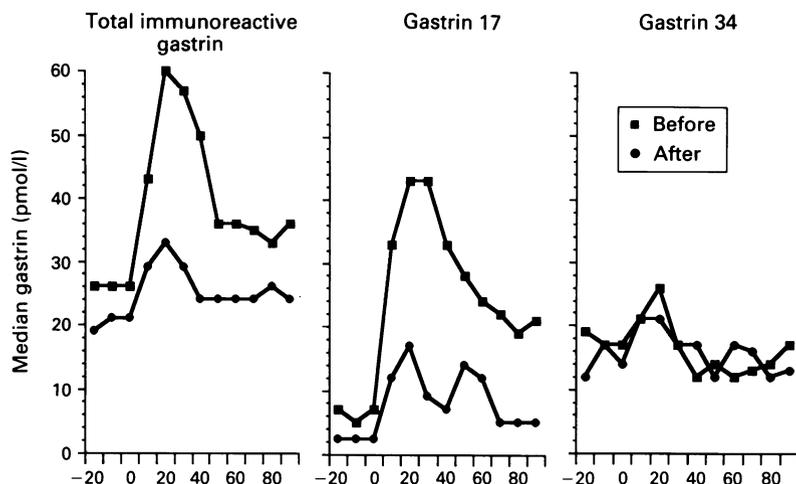


Figure 3: Median serum concentrations of total immunoreactive gastrin (IRG) (R98), gastrin 17 (GP168), and gastrin 34 (subtraction assay) basally and in response to a meal, before and one month after eradication of *Helicobacter pylori*. The meal was begun at time 0. Thirteen patients were studied.

Infirmatory Ethical Committee and each patient gave written informed consent.

Results

The triple anti-*H. pylori* treatment regimen eradicated the infection in each subject. This was confirmed by their 20 minute ^{14}C urea breath test values (percentage of administered ^{14}C urea excreted as $^{14}\text{C}\text{-CO}_2$ per mmol expired $\text{CO}_2 \times \text{kg}$ body weight $\times 100$) which fell from a range of 60–550 before treatment to 2–8 afterwards.

Eradication of *H. pylori* lowered the median basal total immunoreactive gastrin concentration from 26 pmol/l (range 11–43) to 19 pmol/l (8–39) ($p < 0.05$) representing a median fall of 20% (Fig 1). This fall in basal gastrin was the result of a fall in G17 from 6 pmol/l ($< 2.4\text{--}25$) to < 2.4 pmol/l ($< 2.4\text{--}23$) ($p < 0.001$) representing a median fall of 40%. The median basal G34 values were similar before (16 pmol/l, range 0–40) and after (16 pmol/l, range 0–36) eradication of *H. pylori* when estimated by subtraction assay, and also similar before (15 pmol/l, 2–36) and after (10 pmol/l, 2–30) when assayed using antisera R526.

Eradication of *H. pylori* also lowered the postprandial total immunoreactive gastrin concentration with the median 20 minute value falling from 59 pmol/l (38–114) to 33 pmol/l (19–88) ($p < 0.005$), and representing a median fall of 43% (Fig 2). This was again explained by a fall in the G17 concentration from 43 pmol/l (9–95) to 17 pmol/l ($< 2.4\text{--}52$) ($p < 0.001$) representing a median fall of 60%. The median 20 minute postprandial G34 concentration was similar before (26 pmol/l, range 0–57) and after (21 pmol/l, 7–36) eradication of *H. pylori* as determined by subtraction assay, and also similar before (13 pmol/l, 6–42) and after (15 pmol/l, 6–30) as measured by antisera R526.

In both the presence and absence of *H. pylori*, the rise in total immunoreactive gastrin with eating was predominantly caused by an increase in G17 (Fig 3). In the presence of *H. pylori*, the median G17 concentration rose from a basal value of 6 pmol/l ($< 2.4\text{--}25$) to 43 pmol/l (9–95) ($p < 0.0001$) at 20 minute postprandially, and

after eradication rose from < 2.4 pmol/l ($< 2.4\text{--}23$) to 17 pmol/l ($< 2.4\text{--}52$) ($p < 0.0001$). In the presence of *H. pylori*, the median G34 concentration was 16 pmol/l (0–40) basally and 26 pmol/l (0–57) 20 minutes postprandially ($p = 0.2$) as determined by subtraction assay, and 15 pmol/l (2–36) basally and 13 pmol/l (6–42) postprandially measured by antisera R526. After eradication of *H. pylori*, the median G34 was 16.5 pmol/l (0–36) basally compared with 21 pmol/l (5–36) postprandially ($p = 0.06$) as determined by subtraction assay and 10 pmol/l (2–30) basally and 15 pmol/l (6–30) postprandially by antisera R526.

In the fasting state, G17 represented only 23% of the total immunoreactive gastrin in molar terms in the presence of *H. pylori* and this fell to less than 10% after eradication of the infection. In contrast, the 20 minute postprandial total immunoreactive gastrin consisted of 72% G17 in the presence of *H. pylori* and 52% G17 after eradication.

Chromatographic analysis of the postprandial serum from the six patients indicated that G17 and G34 comprised more than 90% of the total immunoreactive gastrin detected by antisera R98 both before and after eradication of *H. pylori* (Fig 4) (Table). Only a minor contribution was made by component I and G14.

Further analysis of the eluate using all three antisera indicated that a minor contribution was made also by a N-terminal 1–13 of G17 identified by the GP168 antiserum. This co-elutes with G14 (detected by R98) (Fig 5). No other aberrant forms were observed.

Discussion

This study indicates that in duodenal ulcer patients circulating gastrin consists almost entirely of G17 and G34 in both the presence and absence of *H. pylori* infection. This is consistent with previous studies which have shown that more than 90% of gastrin in serum consists of G17 and G34 in both duodenal ulcer patients and healthy volunteers.^{23,24} The current study also shows that the increased serum total immunoreactive gastrin in *H. pylori* infection is entirely the result of an increase in G17, with no change in G34. The former is mainly antral in origin, whereas G34 is preferentially released from the duodenum.^{23,25–28} The selective increase in the form of gastrin produced by the antrum is

Chromatographic analysis of postprandial serum gastrin in six patients before and after eradication of *Helicobacter pylori*. Values of the individual gastrins are expressed as pmol/l. The total immunoreactive gastrin the eluate from the G50 column was detected by antisera R98

Patient no	<i>H. pylori</i>	Component I	G34	G17	G14
1	Before	7	127	141	19
	After	1	81	15	2
2	Before	9	76	112	11
	After	7	67	34	5
3	Before	5	38	64	7
	After	4	36	40	5
4	Before	3	55	88	10
	After	3	56	49	7
5	Before	9	106	187	18
	After	8	84	99	11
6	Before	2	48	77	10
	After	1	43	24	6

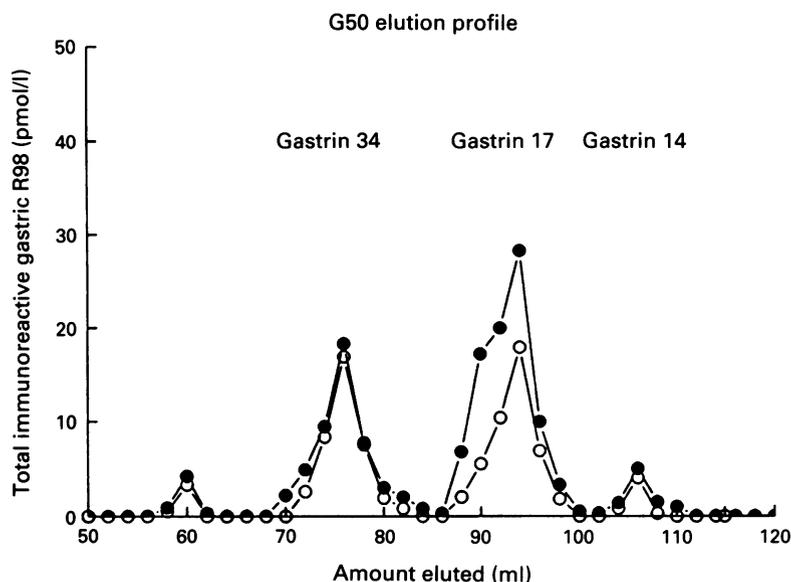


Figure 4: Chromatography of postprandial serum in a representative patient before (●) and after (○) eradication of *Helicobacter pylori*. The total immunoreactive gastrin in the eluate from the G50 column was detected by antisera R98.

consistent with the finding that *H pylori* infection predominantly affects this region of the stomach.

Our finding that fasting serum gastrin consists mainly of G34 is consistent with previous reports.²³⁻²⁹ These earlier studies were performed before the recognition of *H pylori* and most of the patients included would have had the infection. Our present study indicates that in the presence of *H pylori* infection, fasting serum gastrin consists of 77% G34 whereas in the absence of *H pylori* it consists almost exclusively of G34 (98%). It is possible that re-examination of these patients at a later date when any persisting antral gastritis has resolved may indicate that fasting gastrin consists entirely of G34. Our finding also confirms previous reports that postprandial serum gastrin consists of approximately equivalent concentrations of G17 and G34.²³⁻²⁹ The ratio of G17:G34, however, is greater in the presence of the infection (72% G17) than in its absence (52%). The change in the G17:G34 ratio as a result of *H pylori* infection is explained by the infection selectively increasing the G17 concentration.

It has previously been recognised that eating produces a more noticeable rise in G17 than in G34.²³⁻²⁹ We found no statistically significant rise in G34 postprandially, either in the presence or absence of *H pylori*. In contrast, there was a noticeable rise in G17 both before and after eradication of the infection. Our finding is similar to that of Walker *et al*, who reported that the rise in gastrin with eating was solely the result of a rise in G17 in duodenal ulcer patients.²⁹ In an earlier study by Dockray and Taylor, however, a rise in both G17 and G34 was noted after eating in duodenal ulcer patients.³⁰ The reason for these different findings is unclear but may be related to the different forms of meal used.

Most investigators have observed that *H pylori* infection causes a greater percentage increase in the postprandial gastrin than fasting gastrin.²⁻⁶ This can now be explained by the fact that *H pylori* causes a selective increase in G17, which

is the form of hormone which rises most with eating. Though *H pylori* also raises G17 several fold in the fasting state, this only causes a minor increase in fasting serum gastrin which consists predominantly of G34.

The mechanism by which *H pylori* infection stimulates the release of G17 is not known. It was suggested by Levi *et al* that the ammonia produced by the organism's urease would prevent the suppression of antral gastrin release by luminal acid.¹¹ This seems unlikely, however, as earlier studies have shown that neither increasing,⁷ inhibiting,⁸ or completely suppressing⁹ *H pylori* urease activity *in vivo* changes the serum gastrin. In addition, *H pylori* infection has been shown to change gastrin to a similar degree at acidic and neutral pH.¹⁰ The time course of resolution of the hypergastrinaemia during antibacterial treatment indicates that it is more closely related to resolution of the antral gastritis than to suppression of bacterial urease activity.^{9,21}

It has been suggested that the processing of gastrin in duodenal ulcer patients may be altered^{17,18,31,32} and that aberrant forms of gastrin that are immunoreactive but not bioactive may circulate, thus obscuring any correlation

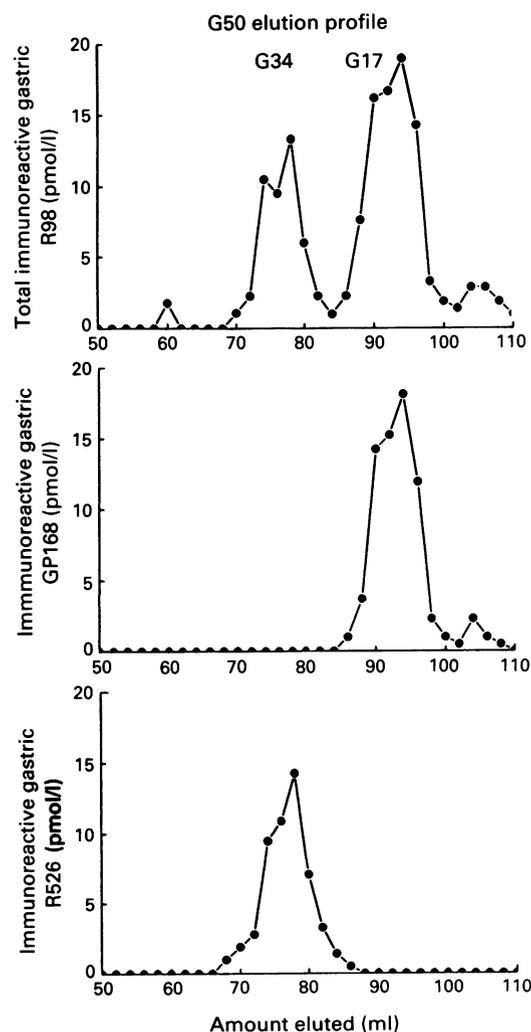


Figure 5: Chromatography of postprandial serum from a *Helicobacter pylori* positive duodenal ulcer patient. The eluate from the G50 column was examined using antisera R98 (total immunoreactive gastrins) GP168 (N-terminal immunoreactive gastrin) and R526 (immunoreactive G34).

between gastrin and gastric acid secretion. Our present study clearly indicates that any contribution made by abnormal forms of gastrin is minimal and that the gastrin which is increased in the presence of *H pylori* infection is G17.

In view of the fact that eradication of *H pylori* infection noticeably lowers postprandial G17 concentrations, one would expect an accompanying decrease in meal stimulated acid secretion. G17 and G34 are equipotent in molar terms with respect to stimulating acid secretion.³³ At one month after eradication of *H pylori* in duodenal ulcer patients we observed a more marked rise in postprandial pH consistent with a reduced acid response to the meal.³⁴ Using intragastric titration, however, Moss *et al*¹² have been unable to show a fall in meal stimulated acid secretion after anti-*H pylori* treatment. The lack of fall in acid secretion despite a considerable fall in biologically active gastrin could be explained by *H pylori* altering the parietal cell sensitivity to gastrin. *H pylori* has been shown to produce a factor which inhibits parietal cells *in vitro*³⁵ and hypochlorhydria may be a feature of the acute infection.³⁶ We have shown, however, that there is no change in parietal cell sensitivity to gastrin after eradication of *H pylori* in duodenal ulcer patients.³⁷

The finding of our present study that eradication of *H pylori* reduces noticeably a biologically active form of gastrin, together with our previous finding that this is not accompanied by reduced parietal cell sensitivity to the hormone, strongly suggests that there should be an accompanying fall in gastrin mediated acid secretion. The failure of studies to show this clearly is probably related to the technical difficulties involved in measuring meal stimulated acid secretion.

This work was supported by a grant from the Biomedical Research Committee of the Scottish Home and Health Department and by the Research Support Group of the Greater Glasgow Health Board. G Mulholland was supported by a grant from the Eastern Health and Social Services Board of Northern Ireland.

The technical assistance of the Western Infirmary Department of Nuclear Medicine, and the secretarial services of Mrs Dorothy Ronney are gratefully acknowledged.

This work was presented at the 1992 Spring Meeting of the British Society of Gastroenterology and published in abstract form in *Gut*.

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