

# Gastrin and somatostatin in *Helicobacter pylori* infected antral mucosa

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## Abstract

***Helicobacter pylori* infection is associated with increased meal stimulated gastrin secretion, but the reason for this is unknown. Sequence specific radio-immunoassays were used to measure the concentration of  $\alpha$ -amidated gastrin, the total progastrin product, and somatostatin in biopsy specimens of human antral mucosa. The antral concentrations of  $\alpha$ -amidated gastrin and of total progastrin products were significantly higher in *H pylori* infected patients than in those not infected by this organism. In contrast, the antral somatostatin concentration was significantly decreased in infected patients. Progastrin processing, determined by gel chromatography, seemed unaffected by *H pylori* infection. The results suggest that the finding of increased gastrin secretion from the antral G cells in *H pylori* infected patients may be a result of reduced inhibition of G-cell secretion by somatostatin.**

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*Helicobacter pylori* infection is associated with increased basal and meal stimulated plasma gastrin concentrations, which become normal after eradication of the bacteria.<sup>1-5</sup> The reason for the increased gastrin secretion is uncertain. The bacterial production of urease, which might cause an increase in the antral surface pH, does not seem to be involved.<sup>5-9</sup> Furthermore, the function of the parietal cells seems to be unaffected by chronic *H pylori* infection, as judged by parietal cell sensitivity to gastrin.<sup>10</sup>

It has recently been suggested that *H pylori* infection inhibits somatostatin synthesis in the gastric mucosa, as the somatostatin-mRNA concentration decreases.<sup>11</sup> Decreased somatostatin production reduces the inhibition of gastric secretory functions.<sup>12 13</sup>

Progastrin processing is less complete during permanent G cell hypersecretion in patients with fundic atrophic gastritis than in normal subjects.<sup>14</sup> In *H pylori* infected patients, the antral gastrin concentration seems increased, as judged by immunohistochemistry.<sup>15</sup>

The present study aimed to examine the influence of *H pylori* infection on the antral expression and processing of progastrin and somatostatin. Since *H pylori* has been shown to inactivate ascorbic acid<sup>16</sup> and decrease the concentration of vitamin C in the gastric juice of infected patients,<sup>17 18</sup> and since ascorbic

acid is a cofactor in the amidation of gastrin,<sup>19</sup> we have also measured antral ascorbic acid and correlated it to the degree of gastrin amidation.

## Methods

### SERUM AND ANTRAL MUCOSAL BIOPSY SPECIMENS

Peripheral venous blood and gastroscopic antral biopsy specimens were taken from 32 fasting patients with dyspeptic symptoms. All had given informed written consent. None of the patients had been taking anti-ulcer treatment in the month before the gastroscopy. Blood was kept at 5°C, and serum was separated within two hours and frozen at -20°C until analysis.

Antral biopsy specimens for measurement of gastrin, somatostatin, and ascorbic acid were frozen within 10 minutes. Tests for urease activity (CLO test, Delta West) were started within five minutes and were performed at 20°C. The medium was read after one and four hours. A patient was regarded as having *H pylori* infection if the CLO test was positive or if *H pylori* could be shown by the histological examination, or both.<sup>20 21</sup> The study was approved by the regional ethical committee.

### HISTOLOGICAL EXAMINATION

Antral biopsy specimens for histological examination were fixed in formalin and routinely processed. Sections were cut at 5-7  $\mu$ m and were stained with Warthing-Starry silver stain in order to show *H pylori*-like micro-organisms.

### MEASUREMENT OF GASTRIN

Frozen antral biopsy specimens were immersed directly in boiling water (pH 6.6), 1 ml per mg tissue. Boiling was continued for 20 minutes, after which tissues were homogenised and centrifuged at 10 000 g for 20 minutes. The supernatants were stored at -20°C until assay.

Bioactive  $\alpha$ -amidated gastrins were measured using antisera 2604 and 2605.<sup>22</sup> Antiserum 2604 binds all bioactive gastrins (sulphated as well as non-sulphated) with equimolar potency, whereas the cross reactivity with cholecystokinin is negligible. Antiserum 2605 has similar characteristics but it binds only non-sulphated gastrin.

Total progastrin products were measured after tryptic cleavage of the extracts using antiserum 8017, specific for the N-terminal

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sequence of human gastrin-17.<sup>23</sup> In this way N-terminally extended precursors are cleaved and the N-termini of gastrin-17 exposed to binding to antiserum 8017.<sup>24</sup> Extracts were incubated with equal volumes of trypsin (2 mg/ml in 0.05 M sodium phosphate pH 7.5) at 20°C for 30 minutes. The enzymatic reaction was terminated by boiling for 30 minutes.

#### MEASUREMENT OF SOMATOSTATIN

Frozen tissues were homogenised in four volumes of acid ethanol<sup>25</sup> and centrifuged at 10 000 g for 20 minutes at 4°C. The extracts were evaporated, pH adjusted to 7.5, and centrifuged again and stored at -20°C until analysis. Antisera R 37 directed against the sequence -Cys-Lys-Asn-Phe-Phe- in somatostatin-14 was produced in rabbits as previously described.<sup>26</sup> Tracer, incubation condition, and separation were as previously described.<sup>27</sup>

TABLE I Characteristics of the patients

H pylori status	No	Age (y) Median (range)	Sex (F/M)	No of patients with peptic ulcer duodenal/gastric
Positive	9	46 (32-88)	6/3	1/3
Negative	23	57 (24-94)	16/7	1/1

#### MEASUREMENT OF ASCORBIC ACID

Antral biopsy specimens were homogenised in metaphosphoric acid and ascorbic acid was measured by high performance liquid chromatography combined with electrochemical detection as previously described.<sup>28</sup> Dehydroascorbic acid was determined after reduction to ascorbic acid.<sup>28</sup>

#### CHROMATOGRAPHY

Gel chromatography was performed on Sephadex G-50 (superfine) columns (1 cm×100 cm) that were eluted with 0.125 M ammonium bicarbonate (pH 8.2) at 4°C with a flow rate of 4 ml/h in fractions of 1 ml. Void and total volumes of the columns were determined using <sup>125</sup>I-albumin and <sup>22</sup>NaCl. The columns were calibrated with gastrin-17 and gastrin-34.

#### STATISTICAL ANALYSIS

The Mann-Whitney U test was used for statistical analysis.

#### Results

Characteristics of the patients are shown in Table I. The age, the distribution between male and female, and the number of patients with peptic ulcer did not differ significantly between the two groups. One *H pylori* negative patient had a duodenal ulcer. This was possibly caused by aspirin taken daily. Only a few patients had duodenal ulcer. They represented a sample of dyspeptic hospital inpatients from a department of medicine.

The concentration of antral bioactive  $\alpha$ -carboxamidated gastrin and the total progastrin products were higher in *H pylori* infected patients than in uninfected patients (Table II). The progastrin processing in the antrum of *H pylori* infected patients was similar to that in uninfected patients - that is, the antrum contained mainly  $\alpha$ -amidated gastrin-17 (Figs 1 and 2), except for a reduced tyrosyl O-sulphation of gastrin in the antral extract from one of the *H pylori* infected patient (Fig 1). The degree of tyrosyl O-sulphation of the  $\alpha$ -amidated gastrins, as judged by the reactivity

TABLE II Concentration (nmol/g) of mature  $\alpha$ -amidated gastrin and total progastrin products in antral biopsy specimens from patients with and without *Helicobacter pylori* infection. Values are median (range)

H pylori status	No	Amidated gastrin	Total gastrin products
Positive	9	13.4 (6.0-32.8)	15.6 (9.2-41.0)
Negative	23	4.6 (0.1-29.3)*	8.6 (0.1-32.4)†

Concentrations were significantly increased in *H pylori* positive compared with negative patients: \*p=0.005; †p=0.011.

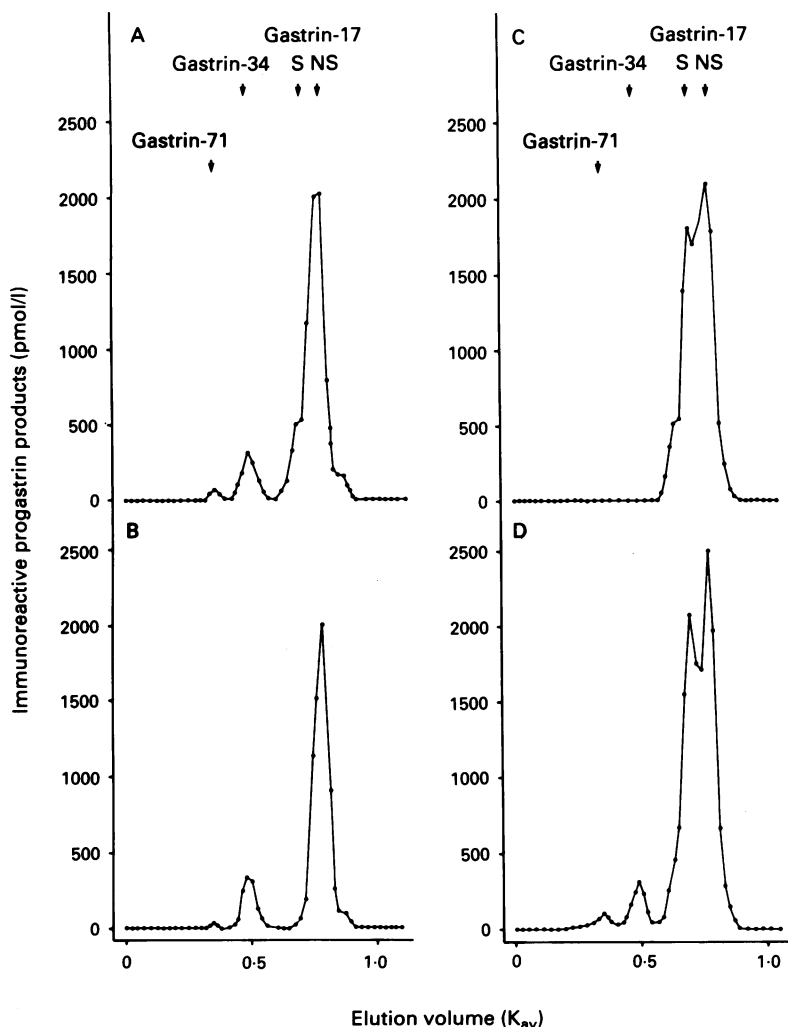


Figure 1: Gel-chromatography of a neutral water extract of an antral mucosal biopsy specimen from a *Helicobacter pylori* infected patient. (A) Shows the elution profile using antiserum 2604 that binds bioactive  $\alpha$ -amidated gastrins with equimolar potency irrespective of the N-terminal chain length and the degree of tyrosyl O-sulphation. (B) Shows the measurements using antiserum 2605, which binds only non-sulphated  $\alpha$ -amidated gastrins. (C) Shows the elution profile using antiserum 8017, which binds the N-terminus of gastrin 17. Finally, (D) shows the measurements using antiserum 8017 but only after incubation of each fraction with trypsin. Thus, in addition to  $\alpha$ -amidated gastrin 17 and C-terminally extended molecules containing the N-terminus of gastrin 17, the measurements in (D) include other progastrin products including gastrin 34.

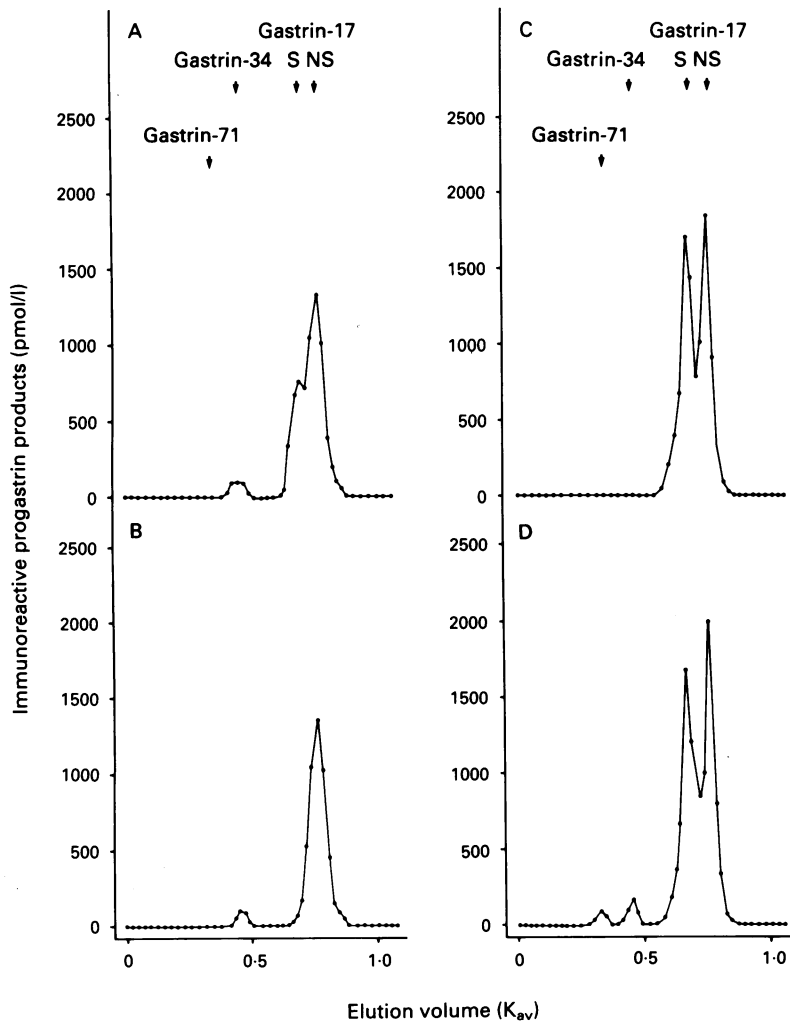


Figure 2: Gel-chromatography of a neutral water extract of an antral biopsy specimen from a patient with dyspepsia, but without *Helicobacter pylori* infection. The measurements are as described in Figure 1.

with antiserum 2605 versus antiserum 2604 was not, however, significantly ( $p=0.21$ ) decreased in infected patients (median=0.61, range 0.50–0.69) compared with uninfected patients (median=0.78, range 0.39–0.69).

The concentration of somatostatin was significantly ( $p=0.05$ ) lower in *H pylori* infected patients (median=0.39 nmol/g, range 0.22–1.44,  $n=9$ ) than in uninfected patients (median=0.91 nmol/g, range 0.21–2.71,  $n=23$ ).

The *H pylori* infection did not increase the oxidation of ascorbic acid to dehydroascorbic acid. In fact, the concentration of ascorbic acid was higher in *H pylori* infected antral mucosa than in non-infected mucosa (Table III).

In serum the concentrations of  $\alpha$ -amidated gastrin and the total progastrin products did

TABLE III Concentration of ascorbic acid and dehydroascorbic acid (nmol/g) in antral biopsy specimens from patients with and without *Helicobacter pylori* infection. Values are median (range)

<i>H pylori</i> status	No	Ascorbic acid	Dehydroascorbic acid
Positive	9	1278 (1023–2318)	98 (28–199)
Negative	23	1017 (500–1983)*	40 (0–420)

Concentration significantly higher in *H pylori* positive compared with negative patients: \* $p=0.03$ .

TABLE IV Concentration (pmol/l) of mature  $\alpha$ -amidated gastrin and total progastrin products in serum from patients with or without *Helicobacter pylori* infection. Values are median (range)

<i>H pylori</i> status	No	Amidated gastrin	Total gastrin products
Positive	9	20 (14–48)	60 (50–86)
Negative	23	21 (11–92)	51 (12–184)

not differ between the two groups (Table IV). Furthermore, no correlation could be shown between antral gastrin or somatostatin concentrations and serum gastrin concentrations.

## Discussion

This study shows that the antral content of progastrin and its products is increased in *H pylori* infected patients. The increase is accompanied by, and perhaps caused by, a corresponding reduction in the antral somatostatin concentration.

The high gastrin concentration in the antrum in patients with *H pylori* infection (Table II) is in agreement with the results in an immunohistochemical study by Sankey *et al.*,<sup>15</sup> who showed a more intense immunostaining of G-cells in the presence of *H pylori* than in uninfected patients.

The increased concentration of bioactive amidated gastrin and of progastrin suggests that the increased concentration is due to an increased gastrin synthesis. This higher synthesis might be due to the inflammation. Thus, tumour necrosis factor  $\alpha$  (TNF- $\alpha$ ), found in high concentration in inflamed antrum,<sup>29</sup> has been shown to increase gastrin gene transcription.<sup>30 31</sup>

The increased meal stimulated gastrin release in *H pylori* infected patients may simply be due to increased gastrin in the G-cells (Table II). A decreased inhibition of the G-cells by somatostatin may also play a part, as we found a lower antral somatostatin concentration in the infected patients (Table III). This is in agreement with Kaneko *et al.*<sup>32</sup> and with Moss *et al.*,<sup>11</sup> who showed a significant rise in somatostatin-mRNA and in somatostatin-immunoreactive cell density after the eradication of *H pylori*. Gastrin release is normally inhibited by somatostatin.<sup>12 13</sup> Finally, the inflammation might also affect the gastrin release as  $\gamma$ -interferon and interleukin-2 stimulates gastrin secretion from dog antrum.<sup>33</sup>

The progastrin processing did not seem to be influenced by *H pylori* infection. Thus, *H pylori* infected antrum, like normal antrum, contained mainly amidated G-17. However, tyrosyl O-sulphation of gastrin was decreased in a patient (Fig 1) with a high antral gastrin concentration similar to patients with hypergastrinemia.<sup>14</sup>

The oxidation of ascorbic acid in antral biopsy specimens was low in infected as well as in uninfected patients. The concentration of ascorbic acid was slightly raised in the antrum of infected patients. This could be due to leukocyte infiltration as the ascorbic acid concentration in leukocytes is higher than in the antral mucosa.<sup>34</sup>

In conclusion, we showed an increased concentration of gastrin and a decreased concentration of somatostatin in the antral mucosa of *H. pylori* infected patients. The increased meal stimulated gastrin release observed in these patients<sup>1-5</sup> may be a result of a higher gastrin content of the antral G cells and decreased inhibition by somatostatin.

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