Quantification of *Helicobacter Pylori* Infection in Gastritis and Ulcer Disease Using a Simple and Rapid Carbon-14-Urea Breath Test

J. C. Debongnie, S. Pauwels, A. Raat, Y. de Meeus, J. Haot, and P. Mainguet

Departments of Nuclear Medicine, Gastroenterology, and Pathology, University of Louvain Medical School, Brussels, Belgium

Gastric urease was studied isotopically in 230 patients with biopsy-proven normal mucosa or chronic gastritis, including 59 patients with ulcer disease. Carbon-14-urea was given in 25 ml of water without substrate carrier or nutrient-dense meal, and breath samples were collected over a 60-min period. The amount of $^{14}CO_2$ excreted at 10 min was independent of the rate of gastric emptying and was not quantitatively influenced by the buccal urease activity. The 10-min $^{14}CO_2$ values discriminated well between *Helicobacter pylori* positive and negative patients (94% sensitivity, 89% specificity) and correlated with the number of organisms assessed by histology. The test was a good predictor of chronic gastritis (95% sensitivity and 96% specificity), and a quantitative relationship was observed between $^{14}CO_2$ values and the severity and activity of the gastritis. In *H. pylori* positive patients, breath $^{14}CO_2$ was found to be similar in patients with and without ulcer disease, suggesting that the number of bacteria is not a determining factor for the onset of ulceration.

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Several reports have described an association of *Helicobacter pylori* with gastric or duodenal ulcer, nonulcer dyspepsia, and gastritis (*1–6*). Direct evidence of a pathogenic role of *H. pylori* was obtained by self-inoculation experiments or by inadvertent iatrogenic transmission followed by acute gastritis and, in some volunteers, the subsequent development of chronic gastritis associated with persistent *H. pylori* infection (*7–9*). Despite the fact that the natural history of chronic gastritis remains unclear, there is mounting evidence that *H. pylori* is the causal agent of most cases of chronic gastritis (*3,6*). In this respect, a clear demonstration of the quantitative relationship between the number of organisms present and the importance of the gastritis would provide additional support for the etiologic role of *H. pylori* in chronic gastritis. Although histologic examination or bacterial culture of biopsies are sensitive diagnostic methods, they are of limited help for accurate quantification of the total bacterial load. The recent development of ^{14}C or ^{13}C-urea breath test (UBT), based on the intense urease activity of *H. pylori* (*10*), could provide the missing quantitative tool since the test integrates the response of the totality of *H. pylori* bacteria active in the stomach (*11–13*).

In the present study, we first evaluated the reliability of the absolute levels of urease activity measured with the ^{14}C-urea breath test. Thereafter, we have used the test to quantitate *H. pylori* infection in a large group of patients with chronic gastritis, searching for a clear positive correlation between the total urease activity and the severity and activity of gastritis. We also have investigated whether the presence of ulcer disease in *H. pylori* positive patients is associated with a further increase of urease activity by comparison with values obtained in absence of ulcer disease.

MATERIALS AND METHODS

Patients referred for upper gastrointestinal endoscopy between March 1988 and March 1989 were considered for entry into the study. Patients with previous gastric surgery, hematologic disease, immunodeficiency, or endoscopic evidence of gastric cancer were excluded. The 266 subjects included in the study underwent a ^{14}C-urea breath test within less than seven days after their endoscopic evaluation. Thirty-six patients were subsequently taken off the study for the following reasons: intake of antibiotics or bismuth containing drugs (n = 9), no antral mucosal biopsies (n = 20), histologic evidence of atrophic gastritis (n = 1), or acute gastritis (n = 6). Patients being treated with antacids (n = 25) or H_2 receptor antagonists (n = 42) were not excluded.

The 230 remaining subjects were classified into five groups, according to the endoscopic findings: duodenal ulcer (n = 37), benign gastric ulcer (n = 22), erosions (n = 42), gastritis (n = 77), and normal (n = 53). On the basis of the histologic results, the subjects were allotted to one of the following groups: normal mucosa (n = 80), chronic gastritis (n = 131), reflux gastritis (n = 16), or lymphocytic gastritis (n = 3).

For the purpose of this study, patients were considered as *H. pylori* positive (HP^+) or *H. pylori* negative (HP^-) on the basis of the presence or absence of the bacteria on biopsy.

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For reprints contact: Dr. S. Pauwels, Centre de Médecine Nucléaire, UCL 54.30, avenue Hippocrate 54, B-1200 Brussels, Belgium.

Endoscopy

Esophagogastroduodenoscopy was performed using lignocain throat anesthesia and intravenous administration of diazepam. The endoscope and biopsy forceps were disinfected in glutaraldehyde. At least two biopsies were obtained from the antrum.

Histology

Biopsy specimens were fixed in Bouin medium and embedded in paraffin. Six micron sections were stained with hematoxylin and eosin, for evaluation of gastritis, and with cresyl violet, for detection of *H. pylori*. Diagnosis of chronic gastritis was based on the presence of lymphoplasmocytes. The severity and activity of the gastritis were graded according to the number of lymphoplasmocytes (graded from 1 to 3) and neutrophils (graded from 0 to 3), respectively. Severity: 1 = only occasional lymphoplasmocytes; 2 = intermediate between grade 1 and 3; and 3 = very dense infiltration. Activity: 0 = absence of neutrophils; 1 = occasional neutrophils; 2 = intermediate between grades 1 and 3; and 3 = dense infiltration. The presence or absence of intestinal metaplasia was also noted. *H. pylori* was searched for at high magnification on the gastric surface, the mucus and in the gastric pits. According to the number of organisms, the severity of *H. pylori* infection was graded from 0 to 3; Grade 0 = no organisms, Grade 1 = only occasional organisms, Grade 2 = intermediate between Grades 1 and 3, Grade 3 = numerous organisms. Reflux gastritis was diagnosed in the presence of foveolar elongation tortuosity and hypercellularity together with oedema, vasodilatation, congestion, and a paucity of inflammatory cells in the lamina propria (*14*). The diagnosis of lymphocytic gastritis was based on the presence of intraepithelial lymphocytes in a ratio of 30 lymphocytes/100 epithelial cells in the areas of maximal lymphocytic infiltration (*15*).

The reproducibility of the histologic evaluation was assessed in 30 patients by taking two sets of two antral biopsies at the same time. These 30 paired biopsies were given a random number from 1 to 60 prior to blind examination by the pathologist. The agreement between the two series of biopsies was evaluated by comparing the histologic scores of gastritis and *H. pylori* infection obtained for each patient in the two series.

Carbon-14-Urea Breath Test

Procedure. After an overnight fast, the patients were given an oral dose of 185 KBq of ^{14}C-urea in 25 ml water. Before and after administration of the isotope, the patients brushed their teeth and rinsed their mouth with water; without swallowing. Breath samples were taken before the test and 5, 10, 15, 20, 30, 45, and 60 min after administration of the labeled compound. All breath samples were collected in counting vials containing 4 ml 0.25 *M* hyamine hydroxyde in ethanol, together with thymolphthalein (60 g/liter) as pH indicator. After addition of 11 ml Aquasol (New England Nuclear Corp., Boston, MA), ^{14}C radioactivity was counted in a liquid scintillation counter.

Calculation of Results. The specific activity was calculated for each sample. The mean specific activity (MSA) was assumed to be the arithmetic mean between the specific activity at the beginning and at the end of a given period. The cumulative $^{14}CO_2$ excretion was calculated by multiplying the MSA by the endogenous output of CO_2 (9 mmol/kg/hr). Breath data were expressed using either the cumulative $^{14}CO_2$ excretion (CE) or the specific activity of a breath sample corrected for body weight (SA). All results of $^{14}CO_2$ activity were expressed as the percentage of the administered dose.

Validation Studies of the UBT. To assess the influence of the urease producing commensal flora in the mouth, 22 patients (11 HP$^+$, 11 HP$^-$) were studied twice the same day. The first test differed from the standard procedure in that the patients were given ^{14}C-urea in 10 ml of water. After keeping the labeled compound in the mouth for 3 min without swallowing, they were asked to spit it out. Eleven patients brushed their teeth and rinsed their mouths with water, whereas the other eleven did not. After completion of the 1-hr breath sampling, the 22 patients underwent a second test following the standard UBT procedure.

To evaluate the influence of the rate of emptying of the liquid meal from the stomach on the UBT values, liquid gastric emptying was studied scintigraphically in combination with UBT in 20 patients (8 HP$^+$, 12 HP$^-$ patients). Following ingestion of 25 ml of water containing 22.2 MBq of ^{99m}Tc-sulfur coiled together with 185 KBq ^{14}C-urea, the patients were positioned erect in front of a scintillation camera. Anterior and posterior views were obtained at 10-min intervals for 1 hr. Regions of interest were drawn around the stomach for each view. Gastric counts were corrected for the physical decay of technetium and for the changes in depth as the meal moves from the fundus to the antrum by use of the geometric mean (*16*). Time-activity curves were generated and the value for 50% emptying time ($t_{1/2}$) was obtained by extrapolation from the curve.

Reproducibility of UBT was studied in 12 patients (6 HP$^+$ and 6 HP$^-$) by repeating the test within three days.

Statistical Methods. All results were expressed as mean ± s.d. Data were analysed using regression analysis, paired and unpaired Student's t-tests as appropriate. Intrasubject variability of UBT was estimated as followed:

$$\text{variation coefficient} = \frac{\text{UBT1} - \text{UBT2}}{\text{UBT1}} \times 100,$$

in which UBT1 and UBT2 corresponded to the UBT values obtained during the first and second study, respectively. The percent differences observed for the 12 patients were expressed as mean.

RESULTS

Assessment of the ^{14}C-Urea Breath Test

Expression of Breath Data. In order to explore whether the sensitivity and specificity of the breath test varies depending on the mode of expression of breath data, we compared 75 controls (*H. pylori* negative on biopsy, normal mucosal histology, no ulcer at endoscopy) with 74 non-ulcer patients with chronic gastritis and *H. pylori* on biopsy. Excretion of $^{14}CO_2$ in breath was found to be significantly higher in these 74 HP$^+$ patients compared to the 75 controls, and this when results were expressed either by the specific activity or by the cumulative excretion over a period varying from 5 to 60 min (Fig. 1). None of the 74 HP$^+$ patients with chronic gastritis had a SA value of the 10 min breath sample ($SA^{10\,min}$) within the range of 95% of the 75 controls (0.14% ± 0.07%). Similar discrimination was obtained using values of cumulative radioactivity or specific activity of breath samples collected between the 15 and 60 min. At 5 min however, three *H.*

pylori positive patients had UBT values within the range of the controls. Therefore, SA^{10min} value was subsequently used for breath data expression. Based on the results obtained in the 75 HP^- patients with normal histology, a SA^{10min} value of 0.3% was considered as the upper limit of normality.

Validation of the UBT. When patients with or without *H. pylori* infection kept the ^{14}C-urea in the mouth for 3 min without swallowing (referred to as buccal test), a large excess of $^{14}CO_2$ was detected within 5 min (4.55% ± 1.8%). The activity rapidly decreased with a mean reduction of 72% between the 5 and 10 min sample. Toothbrushing performed before and after the administration of the labeled compound did not significantly reduce $^{14}CO_2$ exhalation except for the 5 min sample (Fig. 2). Values of breath $^{14}CO_2$ were found comparable in HP^+ and HP^- patients. No correlation was observed between buccal urease activity and the urease activity of the stomach, measured by the standard UBT test, and this with or without toothbrushing.

The 20 patients who underwent a gastric emptying study in association with the ^{14}C-urea test, showed a wide variation in the rate of emptying of the 25 ml of water administered, emptying rate values ranging from 27 to 120 min. Similar values of t½ were observed in HP^- (51 ± 28 min) and HP^+ patients (49 ± 25 min; $p > 0.8$) whereas the difference in UBT values between those two groups was statistically significant ($HP^- = 0.15\% \pm 0.06\%$; $HP^+ = 3.09\% \pm 2.08\%$; $p < 0.001$). No correlation was found between the gastric emptying rate and UBT values.

When 12 patients (6 HP^+, 6 HP^-) were studied twice within three days, no statistical difference was observed between the two sets of UBT studies (2.39% ± 2.79% versus 2.52% ± 2.82%). Although the mean day-to-day coefficient of variation was relatively high (24%), none of the HP^- patients had SA^{10min} values exceeding 0.3% and inversely none of the HP^+ patients fell within the normal range.

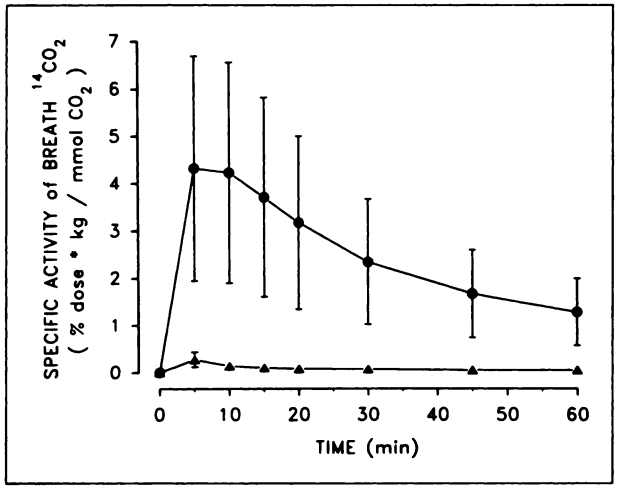

FIGURE 1. Breath $^{14}CO_2$ specific activity after oral administration of ^{14}C-urea in 75 HP^- patients with normal histology (▲–▲) and 75 HP^+ patients with histology proven chronic gastritis and no ulcer at endoscopy (●–●). Each point represents mean ± s.d.

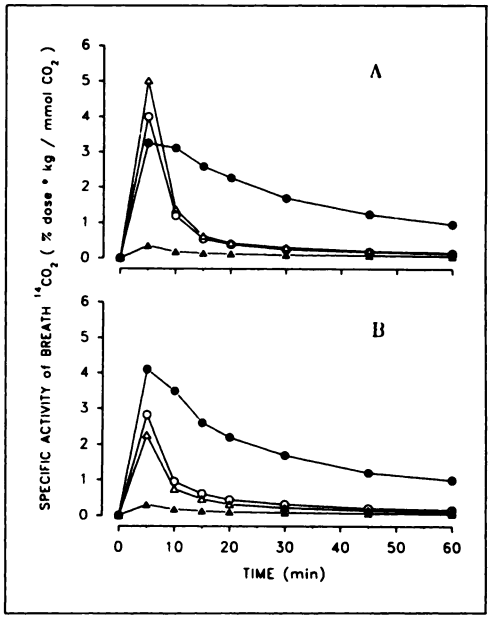

FIGURE 2. Comparison of buccal urease breath test (open symbols) and standard gastric UBT (closed symbols) in 11 HP^- (△, ▲) and 11 HP^+ patients (○, ●). The tests were performed with toothbrushing (B) or without toothbrushing (A). To quantitate buccal urease activity, patients kept the ^{14}C-urea in mouth during 3 min and spat it out without swallowing. Breath samples were collected during 60 min. The same day the patients were restudied using the standard UBT procedure. Each point represents the mean value of breath specific activity.

Reproducibility of Histologic Scoring

The analysis of the histologic findings obtained in the 30 paired biopsy specimens, revealed that in 77%–87% of the cases the scores for *H. pylori* infection, severity and activity of gastritis were identical in the two series of mucosal samples (Table 1). Major discrepancy between the two series was observed in one case, considered as Grade 3 of activity in one set of biopsies and Grade 0 in the other set. Six patients were considered as HP^- in one series of biopsies, and HP^+ in the other series; in five of these cases the bacterial load was however very low (Grade

TABLE 1
Reproducibility of Histologic Grading of *H. pylori* Infection, Severity, and Activity of Gastritis in 30 Paired Biopsy Specimens*

	Identical score	1 grade difference	2 grade difference	3 grade difference
H. pylori	23 (77%)	6 (20%)	1 (3%)	—
Severity	23 (77%)	6 (20%)	1 (3%)	—
Activity	26 (87%)	3 (10%)	—	1 (3%)

* Values give the number of cases in which the histologic scores obtained in paired biopsies were identical or revealed a 1, 2 or 3 grade difference between the two paired biopsies.

TABLE 2
Breath Test, Histology, and Endoscopy Results in Eleven False-Positive and 8 False-Negative Results of Breath Tests

Patient	Breath test SA 5 min %	Breath test SA 10 min %	Endoscopy	Histology	Histology grades Severity	Activity	HP*
False +ve UBT							
1	2.91	2.92	gastritis	chronic g.	2	1	0
2	2.63	2.29	gastric ulcer	reflux g.	0	0	0
3	1.25	1.53	normal	chronic g.	1	2	0
4	1.90	1.37	duodenal ulcer	chronic g.	1	0	0
5	0.75	1.25	gastritis	reflux g.	0	0	0
6	1.14	1.12	gastric ulcer	chronic g.	2	0	0
7	0.57	0.81	gastritis	chronic g.	1	0	0
8	0.74	0.62	gastritis	chronic g.	1	0	0
9	0.53	0.46	duodenal ulcer	reflux g.	0	0	0
10	1.07	0.38	erosions	normal	0	0	0
11	0.56	0.31	normal	normal	0	0	0
mean	1.01	1.19					
s.d.	0.69	0.79					
False −ve UBT							
1	0.06	0.04	normal	normal	0	0	1
2	0.09	0.05	gastritis	normal	0	0	1
3	0.11	0.07	normal	normal	0	0	1
4	0.23	0.15	gastric ulcer	normal	0	0	1
5	0.23	0.17	gastritis	normal	0	0	1
6	0.23	0.18	normal	reflux g.	0	0	1
7	0.33	0.20	erosion	reflux g.	0	0	1
8	0.54	0.28	normal	chronic g.	1	1	1
mean	0.23	0.14					
s.d.	0.15	0.08					

* HP = Helicobacter Pylori

1) in the positive biopsies. Diagnostic disagreement between the two sets of biopsies was observed in three additional cases: in two cases, chronic inactive gastritis with Grade 1 severity was observed in one set and not present in the other, and in one case the gastritis was found to be active in the first series of biopsies and not in the other.

Urease Activity and Bacterial Load

On the basis of histology, 127 patients were HP^+, and 103 were HP^-. UBT was positive in 119 of the 127 HP^+ patients and negative in 92 of the 103 HP^- patients. Thus, compared to histology, UBT appeared to have a sensitivity of 94%, and a specificity of 89% for detection of *H. pylori* infection. It is noteworthy that the eight false-negative breath tests were all obtained in patients with Grade 1 of bacterial load, and in five cases the gastric mucosa was normal on biopsy (Table 2). Endoscopic examination of the 11 HP^- patients with false-positive UBT, revealed the presence of a duodenal or gastric ulcer in four patients. In addition, histologic findings indicated the presence of chronic gastritis in six of these 11 HP^- patients. In these eleven cases of false-positive UBT, the mean breath $^{14}CO_2$ exhaled at 5 min was lower than the mean value obtained at 10 min, indicating that the elevated SA^{10min} values can not be attributed to a particularly elevated buccal urease activity. Taken together these results suggest that five false-negative and six false-positive UBTs could correspond to false histologic readings so that the true sensitivity and specificity of UBT would reach 98% and 95%, respectively.

The amount of $^{14}CO_2$ excreted at 10 min correlated with the number of *H. pylori* organisms assessed semiquantitatively ($p < 0.001$) by histology (Table 3). The mean UBT value increased significantly as the colonization score in-

TABLE 3
Carbon-14-Urea Breath Test and Histologic Grading of Bacterial Load in 230 Patients

	Grade 0 (n = 103)	Grade 1 (n = 48)	Grade 2 (n = 46)	Grade 3 (n = 33)
		%		
mean	0.25	2.64*	4.28†	4.81
s.d.	0.42	2.25	2.28	2.06
range	0.02–2.92	0.04–8.38	0.66–9.04	0.98–8.16

* $p < 0.001$ compared to grade 0 patients
† $p < 0.001$ compared to grade 1 patients

creased from Grade 0 to Grade 1, and from Grade 1 to Grade 2. Although patients with Grade 3 colonization excreted more $^{14}CO_2$ than Grade 2 patients, the difference did not reach statistical significance ($p < 0.1$).

Urease Activity and Chronic Gastritis

In the 80 patients with normal mucosa, proven by histology, SA^{10min} values remained very low, ranging from 0.02% to 0.36% with a mean value of 0.14% ± 0.07%; only three patients had a value exceeding 0.3%. On the basis of the histologic evaluation, 75 of the 80 patients had no *H. pylori* infection, and in the five remaining patients only very rare organisms were observed. Results of breath test were similar in both subgroups (0.16% ± 0.11% in HP^+ and 0.14% ± 0.07% in HP^-).

Patients with reflux (n = 16) or lymphocytic gastritis (n = 3) had UBT values within the normal range except for three patients with reflux gastritis.

The mean UBT value observed in the 131 patients with chronic gastritis (3.74% ± 2.38%) was significantly increased ($p < 0.001$) compared to the patients with normal gastric mucosa. Moreover, in 124 patients, breath $^{14}CO_2$ exceeded 0.3%. In six of the seven patients with normal UBT value, no *H. pylori* organisms were detected on biopsy and gastritis was classified as mild and inactive.

A positive correlation was observed between urease activity measured by UBT and the histological estimate of the number of lymphoplasmocytes or neutrophils infiltrating the antral mucosa (Table 4). Thus, mean UBT value increased significantly from 2.36% ± 2.30% to 3.91% ± 2.14% as the number of lymphoplasmocytes increased from Grade 1 to Grade 2 of severity ($p < 0.005$). A further increase in urease activity was found when patients with Grade 3 severity (SA^{10min} 4.35% ± 2.48%) were compared to patients with Grade 2. The difference was, however, not statistically significant ($p = 0.34$). Similar results were obtained for neutrophils, in that an increase in UBT values was observed as the activity score increased from Grade 0 (1.13% ± 1.42%) to Grade 1 (3.03% ± 2.05%; $p < 0.005$), from Grade 1 to Grade 2 (4.10% ± 2.26%; $p < 0.05$) and from Grade 2 to Grade 3 (5.02% ± 2.13%; $p = 0.07$).

Urea Activity and Ulcer Disease

In 50 of 59 patients with ulcer disease, UBT values at 10 min were above 0.3%. The rate of abnormal breath test was similar in the 37 patients with duodenal ulcer (84%) and with gastric ulcer (86%). The frequency of positive UBT was significantly lower in patients with endoscopic evidence of gastritis (55%) or erosions (45%), and in patients with normal endoscopy (38%).

When the 127 HP^+ patients were classified into five groups according to the endoscopic findings, there was no significant difference in the mean $^{14}CO_2$ excretion between these five groups (Table 5). The mean severity or activity scores of gastritis were also similar in these five endoscopic diagnostic groups. Thus, the antral gastritis was not more severe or more active in duodenal or gastric ulcer patients than in patients without peptic ulcer disease.

DISCUSSION

Recently, the ^{14}C-urea breath test has been proposed as a noninvasive method for the diagnosis of *H. pylori* infection (*11,12*). In this context, the sensitivity and the specificity of the test have been extensively studied by comparison with other methods able to detect the presence of the bacteria. Although the screening and follow-up value of the breath test is widely accepted, very few studies have been devoted to the validation of the quantitative value of the breath test. It is clear that several factors may influence the amount of $^{14}CO_2$ excreted, so that the reliability of the values obtained remains questionable. Indeed, it is often assumed that the urease-producing commensal flora from the mouth may give rise to falsely increased levels of breath $^{14}CO_2$. In addition, it has been thought that any variation of the gastric emptying rate may modify the enzyme-substrate contact time, and thereby influence the absolute amount of urea hydrolyzed by the bacteria in the stomach (*17*). Our results suggest that these assumptions are unjus-

TABLE 4
Carbon-14-Urea Breath Test and Histologic Severity and Activity Scores of Gastritis in 131 Patients with Chronic Gastritis

	Severity of gastritis			Activity of gastritis			
	Grade 1 (n = 25)	Grade 2 (n = 67)	Grade 3 (n = 39)	Grade 0 (n = 13)	Grade 1 (n = 33)	Grade 2 (n = 55)	Grade 3 (n = 30)
				%			
mean	2.36*	3.91†	4.35	1.13	3.03‡	4.10§	5.02
s.d.	2.30	2.14	2.48	1.42	2.05	2.26	2.13
range	0.08–6.94	0.12–8.38	0.63–9.04	0.10–5.70	0.08–6.61	0.66–8.38	1.00–9.04

* $p < 0.001$ compared to patients with normal mucosa (Grade 0 of severity; SA^{10min} 0.14% ± 0.07%).
† $p < 0.005$ compared to patients with Grade 1 of severity.
‡ $p < 0.005$ compared to patients with Grade 0 of activity.
§ $p < 0.05$ compared to patients with Grade 1 of activity.

TABLE 5
Carbon-14-Urea Breath Test and Histologic Scores of Gastritis in 127 HP^+ Patients Classified According to the Endoscopic Findings*

Endoscopic diagnosis	UBT	Histologic scores for gastritis	
		Severity	Activity
	%		
Duodenal ulcer (28)	3.87 ± 2.08	2.29 ± 0.52	1.86 ± 0.74
Gastric ulcer (18)	3.32 ± 2.35	2.47 ± 0.61	2.18 ± 0.78
Erosions (19)	3.60 ± 2.71	2.06 ± 0.78	1.94 ± 0.85
Gastritis (40)	3.95 ± 2.21	2.11 ± 0.68	1.82 ± 0.82
Normal (22)	3.99 ± 2.78	2.11 ± 0.57	2.00 ± 0.75

* Results are expressed as mean ± s.d. values obtained in each group of patients.

tified. Although a wide variation in gastric emptying rate was observed, no relationship was found between the half-time of emptying of the liquid meal and the magnitude of the metabolism of the labeled urea. To the best of our knowledge, no previous study included a simultaneous measurement of the rate of gastric emptying and hydrolysis of the labeled urea.

In standard test conditions, the radiolabeled compound is ingested by the patient in one or two swallows, so that the mouth transit time is very short. When patients were asked to keep the tracer in their mouths for 3 min, a large excess of $^{14}CO_2$ was detected in the 5-min sample. This early peak of $^{14}CO_2$ was found to be similar in HP^+ and HP^- patients and decreased very rapidly over the next 5 min. Comparison of the results obtained with the buccal test and the standard UBT test confirmed that, except for the 5 min breath sample, the buccal urease activity had no influence on the amount of $^{14}CO_2$ detected in breath during the standard UBT test. Our results also showed that washing out the mouth of the patient prior to examination had a minor effect on mouth urease activity.

In a recent review of the diagnostic possibilities for *H. pylori* infection, Graham and his colleagues (*17*) insist on the importance of giving ^{14}C-urea mixed with cold urea and the use of a nutrient-dense meal. If these two elements of the test are not respected, the test would be considerably less accurate for the diagnosis of *H. pylori* infection. This idea was supported by the report of Tytgat and co-workers (*13*) who found that when UBT was carried out with a nutrient-dense meal, the values of breath $^{14}CO_2$ in HP^+ patients exceeded those obtained without a test meal whereas the addition of a test meal had no effect on the 2 HP^- patients studied. Although the Amsterdam group concluded that the use of a test meal increased the resolution of the breath data, they found no effect of the test meal on the ability of UBT to identify HP^+ patients. In the present study, labeled urea was given without a carrier- or nutrient-dense meal. This simplification of the test was not accompanied by a loss of accuracy. A further advantage of the present method is a significant shortening of the sampling period. A single breath sample taken 10 min after ingestion of the isotope separated HP^+ and HP^- patients with a 98% sensitivity and a 95% specificity, values that are similar to those reported by the other groups (*11,13*), and better than those we previously obtained with other diagnostic methods (*18,19*).

Our results demonstrate a clear relationship between the overall gastric urease activity and the number of bacteria found on sections of antral mucosa, at least for Grades 0 to 2, suggesting that UBT is a quantitative index of the bacterial load. The same relationship between UBT and the number of bacteria assessed by culture was reported by Rauws (13) with the same limitation: no difference could be found between Grades 2+ and 3+ of colonization. Since an adequate mass of carrier urea was used by the Amsterdam group, it is unlikely that the lack of discrimination between patients with Grades 2 and 3 of bacterial load is due to an exhaustion of the substrate. Other investigators comparing the time of reaction of a rapid urease test (CLO test) and the semiquantitative histologic assessment of *H. pylori* reported conflicting results: no close correlation was found by Börsch (*20*), an insignificant trend was noted by Marshall (*2*), whereas a quantitative relationship was suggested by Hazell (*1*).

The high frequency of *H. pylori* infection in patients with chronic gastritis was confirmed in the present study. Excluding reflux and lymphocytic gastritis, urease activity was found abnormal in 95% of patients with histologically proven chronic gastritis and normal in more than 95% of patients with gastritis. Therefore, UBT may be proposed as a sensitive and specific test to predict the presence of antral gastritis. The interrelation between *H. pylori* and active chronic gastritis is well established (*3,21–24*), and recent reports suggest a positive correlation between the degree of bacterial infection and the grades of activity of the gastritis (*1,25*). In contrast, the association between the bacterial load and the degree of severity is more controversial (*1,26*). We found a quantitative relationship between the amount of urea metabolized and the Grades (0 to 2) of both activity and severity of the gastritis. A similar relationship was observed by Hazell who measured the urease activity on gastric mucosa biopsies. Although the highest UBT values were obtained in patients with Grade 3 of inflammation, the breath test could not distinguish patients with Grade 3 from those with Grade 2 in our study. This could be due to a true limitation of the methods used or it may suggest that factors other than bacterial colonization are involved in the aggravation of the gastritis from Grade 2 to Grade 3.

The high prevalence of *H. pylori* infection in patients with peptic ulcer has raised the question of the pathogenecity of the bacteria in gastric and duodenal ulcers. Recent observations that eradication of *H. pylori* could significantly reduce the relapse rate of peptic ulcers pro-

vided strong, but still indirect, evidence for a cause-effect relationship between *H. pylori* colonization and ulcer disease (*27*). If most of the infected patients have gastritis, only a limited number develop ulcers, and the sequence of events leading some of these patients after *H. pylori* infection to ulceration is poorly understood. In this context, we have examined whether the occurrence of gastric or duodenal ulcer could be associated with a particularly increased number of *H. pylori* organisms. Our results showed that most of ulcer patients had a positive UBT. However, the urease activity measured in the HP^+ ulcer patients was similar to that found in HP^+ nonulcer patients, indicating that the bacterial load was not increased in ulcer compared to nonulcer patients. The present observations extend the previous study of Queiroz and coworkers who noted no difference in the intensity of bacterial growth, assessed on biopsies, between gastritis patients with and without duodenal ulcer (*28*).

Taken together, these findings suggest that the role of *H. pylori* in the pathogenesis of peptic ulcers presents some similarity with the role played by acid. Indeed, both factors have to be present for the onset of ulceration, and their suppression is associated with healing and prolonged remission of the ulcer. Although the presence of acid constitutes a prerequisite, the amount of acid, however, does not have to be increased. Similarly, *H. pylori* organisms have to be present, but their number appears not to be a determinant factor for the onset of the ulceration.

The present results are of interest because they allow us to propose a simple and rapid noninvasive method for accurate detection of *H. pylori* infection. The quantitative relationship found between the urease activity, measured by UBT and both activity and severity grades of gastritis provides further support to the pathogenic role played by the bacteria in chronic and active gastritis. The evidence that in HP^+ patients, the presence of ulcer disease is not associated with an increased urease activity compared to nonulcer gastritis suggests that the importance of the bacterial load is not a determinant factor in the pathogenesis of ulcer disease.

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