Carbon-14 Urea Breath Test for the Diagnosis of Campylobacter Pylori Associated Gastritis

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Urease in the human gastric mucosa is a marker for infection with *Campylobacter pylori* (CP), an organism suspected of causing chronic gastritis and peptic ulceration. To detect gastric urease, we examined 32 patients who were being evaluated for possible peptic ulcer disease. Fasting patients were given 10 μCi (370 kBq) of ^{14}C-labeled urea. Breath samples were collected in hyamine at intervals between 1 and 30 min. The amount of ^{14}C collected at these times was expressed as: body weight × (% of administered dose of ^{14}C in sample)/(mmol of CO_2 collected). The presence of *C. pylori* colonization was also determined by examination of multiple endoscopic gastric biopsy specimens. On average, patients who were proven to have *C. pylori* infection exhaled 20 times more labeled CO_2 than patients who were not infected. The difference between infected patients and *C. pylori* negative "control" patients was highly significant at all time points between 2 and 30 min after ingestion of the radionuclide ($p < 0.0001$). The noninvasive urea breath is less expensive than endoscopic biopsy of the stomach and more accurate than serology as a means of detecting *Campylobacter pylori* infection. Because the test detects actual viable CP organisms, it can be used to confirm eradication of the bacterium after antibacterial therapy.

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Campylobacter pylori (CP) produces large amounts of urease (urea-amidohydrolase), an enzyme not present in mammalian cells and absent from the normal human gastric mucosa (*1,2*). The new bacterium may be the cause of type B gastritis (*3–5*), an inflammatory condition of the stomach thought by some to cause peptic ulceration (*6*). Other urease-producing bacteria rarely colonize the stomach, so tests for gastric urease are specific and sensitive detectors of CP infection, and thus of gastritis. This paper describes a simple breath test to detect gastric urease.

PATIENTS AND METHODS

Evaluation of the test was performed on 32 consecutive patients (23 men, 9 women) referred to our institution's ulcer research clinic. At upper gastrointestinal endoscopy, every patient had four biopsy specimens taken from the prepyloric antral mucosa, two for histopathology, one for gram-stain and culture, and one for a rapid urease test (see following). Biopsy specimens were processed in a manner previously described (*7*). For the purposes of this study, *C. pylori*-positive patients were defined as those with characteristic bacteria detected on gram-stained smear, culture, or histology (*5*).

For the rapid urease test (*2*), a biopsy specimen was inserted into a yellow gel ("CLOtest") containing urea and a pH indicator. Urease was present if a red color change occurred as a result of the production of ammonia from the hydrolysis of urea. When the CLOtest was positive, the time taken for the color change to occur was recorded by the endoscopist. These data were collected to see whether the rapid urease biopsy test and the breath test were equivalent means of quantitating gastric mucosal urease.

Nonessential medications were omitted during the 12 hr before the breath test, and patients were excluded from the study if they had taken antibiotics or bismuth-containing drugs during the previous 28 days [medications that could suppress CP infection (*5*)]. The breath test was always performed within 7 days of an elective endoscopy (either before or after). Clinical data, and microbiologic, histologic, and breath-test results for individual patients were collected independently and were not collated until evaluation of data from each patient was complete. The protocol was approved by the Royal Perth Hospital Human Rights Committee and all patients gave written informed consent for the test.

The principle of measuring carbon-14 (^{14}C) as carbon dioxide excretion in the breath was adopted from previous reports (*8–10*). Carbon-14-labeled urea was supplied as a freeze-dried

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ampoule. The contents of the ampoule 250 μCi (9.25 MBq) were dissolved in sterile water and made up to 100 ml. This resulted in a stock solution of 2.5 μCi (92.5 kBq/ml), that was stored at 2° to 4°C before use. The patient dose was gravimetrically dispensed from the stock solution so that 370 kBq (10 μCi) of radioactivity could be administered to the patient in 25 ml of water. At the same time a 1:125 dilution of the stock solution was prepared as a standard for liquid scintillation counting.

Patients fasted for at least 6 hr, usually overnight, before the test. To diminish contamination from urease-producing commensal flora in the mouth, patients brushed their teeth with water only, that was not swallowed. The patient then gave a control breath sample before drinking the isotope. After administration of the isotope the patient again cleaned his teeth and rinsed his mouth over a washbasin with the water running. For each sample, the patient exaled through a short corrugated tube connected to a chamber of calcium chloride granules as a drying agent (Fig. 1). The breath then bubbled into a 20-ml scintillation counter vial containing 0.5 mmol of hyamine dissolved in 2 ml of ethanol. The breath sample was complete when a pH indicator (thymolphthalein) in the hyamine solution changed from blue (alkaline) to colorless (acid). Most patients were able to change the color of the hyamine solution with a single long breath, thus decreasing contamination of the breath from urea hydrolyzed in the oropharynx. In retrospect, the drying step could have been omitted, but the drying chamber ensured that patients could not accidentally inhale the hyamine solution.

Samples were obtained at baseline, and at 1, 2, 5, 10, 15, 20, 25, and 30 min after the patient had drunk the isotope. During the test, patients were allowed to move around, but they usually sat upright reading magazines. Whenever possible, a 24-hr urine sample was collected after the test.

After addition of 10 ml of scintillation fluid, standards were counted in duplicate in a liquid scintillation counter. Counts were corrected to disintegrations per minute (DPM) using an external standard, quench program. Samples were counted to a 1% coefficient of variation and results were expressed as

$$(\text{DPM/mmol } CO_2 \text{ collected}) \times 100 \times \text{body-weight (kg)}/(\text{DPM administered}), \text{ and}$$
$$(\text{DPM/ml urine}) \times \text{urine volume} \times (100/\text{DPM administered}).$$

The breath radioactivity was multiplied by body weight to correct for the influence of endogenous CO_2 production on the breath-specific activity of ^{14}C (7). The calculation gave a result that was independent of the ^{14}C dose administered, the amount of CO_2 collected, and the weight of the patient.

An example of a calculation from a positive patient is given below:

Age = 60 Sex = M

DPM of standard = 176,000;
Proportion of dose in standard = 1/125;
Weight of patient = 180 lb = 180/2.2 kg;
Concentration of hyamine = 0.25 m*M*;
Volume of collecting solution = 2 ml;
DPM of sample taken 10 min after dose = 6433.

$$\text{result } \frac{6433 \times 100 \times 180/2.2}{(176000 \times 125) \times (2 \times .25)} = 4.78.$$

In CP-negative patients DPM values of 300 are expected after 10 min (result of 0.37 for the demonstration patient given above). Baseline samples in most patients were around 100 DPM with a background of ~70 DPM.

When the 32 patients described above had been examined by both breath test and biopsy, the breath test was used as an accessory means of diagnosis in 40 additional patients who were undergoing evaluation for dyspepsia. These patients could not be included in the formal study because they either did not have a gastric biopsy taken, or they had recently received antibacterial therapy.

RESULTS

Of the 32 patients who took part in the formal evaluation study, 16 were CP+ and 16 were CP−. Clinical data on these patients are given in Table 1.

Table 2 gives detailed breath-test results for these patients. It demonstrates a significant difference between the CP+ and CP− groups at time points after 1 min ($p < 0.0001$, t-test). Graphic comparison of the two groups is shown in Figure 2.

In CP+ patients the maximal urease activity was in the stomach so the $^{14}CO_2$ increased and remained high after the urea was swallowed. In the CP− persons, however, the maximal urease activity was in the mouth and the esophagus. In this group the peak $^{14}CO_2$ excretion was immediately after ingestion with a subsequent decline as the urea entered the sterile acid environment of the stomach.

As shown in the figure, the standard deviation was wide for both curves, but at 10 min any value >1.5 was CP+ and any value <0.5 was CP−. In the CP− group the highest values were from a patient with deformity of the pyloro-duodenal segment who had previously undergone pyloroplasty and vagotomy. In this patient there may have been some other urease-producing bacterial flora in the stomach as a result of diminished acid secretion and gastric stasis. Bacteria (not *C. pylori*) were seen adhering to the mucosa in the histologic sections on this patient. His breath-test results at 5, 10, and 15 min were 1.26, 1.11, and 0.99.

In the CP+ patients breath-test values at 5, 10, and 15 min were all above 1.5. Although there was no overlap with the CP− group in this study, the relatively small number of patients examined causes the confidence intervals for each group to be wide so that both CP− and CP+ patients may be found in the region between 0.75 and 0.92. Therefore, there may be some patients who give an indeterminate result, i.e., values between 0.70 and 1.5 at times 10 and 15 min. In the majority of patients, however, a single sample at 15 min will provide adequate data.

Twenty-four-hour urine collections were obtained from 11 CP− and 13 CP+ patients (Table 3). The amount of ^{14}C (presumed to be in urea) excreted in the CP− patients was 67% of the administered dose (s.d.

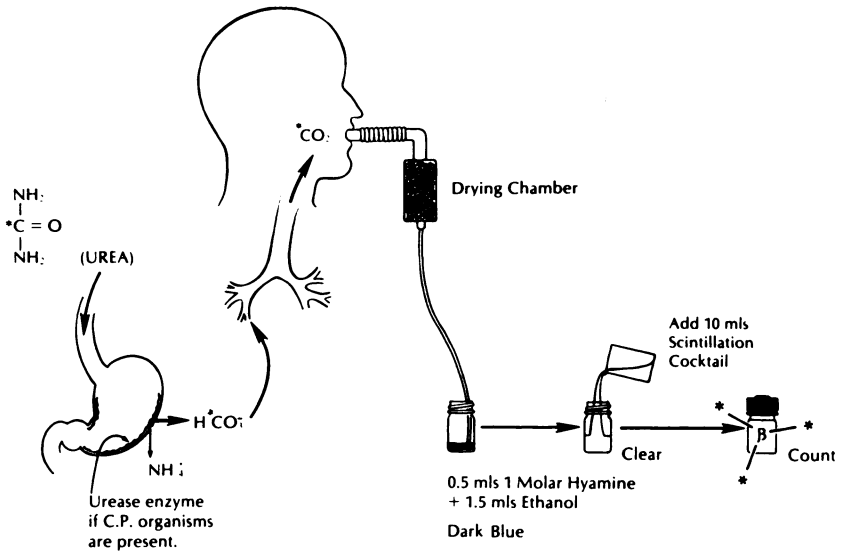

FIGURE 1
Diagram showing apparatus used to perform the test. In subsequent studies the drying chamber has been omitted and patients have blown through a disposable 1-ml plastic pipette.

13, range 47–93%); and in the CP+ patients was 42% of the administered dose (s.d. 12, range 14–65%). These differences were again highly significant ($p < 0.0001$ t-test).

In all 32 patients, the presence of *C. pylori* organisms was also determined by the rapid urease biopsy test performed at the time of endoscopy. For the 16 CP+ patients, the average time taken for the biopsy urease test to change color was 4 min, (range 1–10 min) but there was no correlation between the reaction time of the biopsy urease test and ^{14}C CO_2 excretion detected with the breath test.

Figure 3 shows individual breath-test results for the 40 patients who could not be included in the evaluation study (44 tests, four patients were tested on two occasions). To improve the resolution of the curves, a logarithmic scale has been used on the Y axis. Twenty-one patients had large amounts of gastric urease (A) and 14 patients had no gastric urease (B). Separate from these groups was a small subgroup of patients in the low-positive and high-negative range (C). Most of these were patients who had forgotten their instructions and had taken antibiotic medication or bismuth in the week of the test, or had taken cimetidine in the 24 hr before the test. For example, one patient was taking 250 mg of amoxycillin daily until the day before his test which gave a result in the high negative range (*1). After ceasing his antibiotic we repeated the test 2 wk later by which time gastric urease activity had increased threefold (*2) indicating probable recrudescent CP infection.

There were 57 patients in whom a 24-hr urine collection was performed after taking the isotope. There was a strong correlation ($r = -0.71$) between ^{14}C excretion in the urine and the log of the maximum $^{14}CO_2$ breath value. The urinary ^{14}C excretion assisted in radiation dosimetry calculations.

TABLE 1
Characteristics of 32 Patients in the Formal Evaluation Study

	Number	Mean age (yr)	Men	Women	Duodenal ulcer	Gastric ulcer	Other
CP−*	16	45	14	2	9	1	6
CP+†	16	46	9	7	14	0	2

* CP− = *C. pylori* not detected by culture, histologic examination or Gram stain of gastric antral biopsy specimens.
† CP+ = *C. pylori* detected by any one of these tests.

Dosimetry

Ingested urea may pass unaltered into the urine or may be hydrolyzed by bacteria through the following

TABLE 2
Breath Test Results on 32 Patients

Time (min)	No of pts.	Mean	s.d.	Minimum	Maximum
Negative findings for *C. pylori* (16 patients)					
1	11	0.84	0.50	0.15	1.69
2	11	0.55	0.39	0.04	1.39
5	13	0.30	0.30	0.02	1.26
10	13	0.22	0.30	0.02	1.10
15	16	0.20	0.32	0.01	1.03
20	12	0.28	0.39	0.01	1.26
25	12	0.33	0.43	0.01	1.40
30	16	0.27	0.40	0.01	1.50
Positive findings for *C. pylori* (16 patients)					
1	13	1.81	1.10	0.24	3.50
2	13	3.73	1.34	1.53	5.82
5	15	4.30	1.67	1.66	7.80
10	15	4.38	1.80	1.83	7.90
15	16	3.97	1.59	1.65	6.21
20	14	3.11	1.34	1.32	4.96
25	14	2.58	1.13	1.16	4.22
30	16	2.36	1.07	0.96	4.13

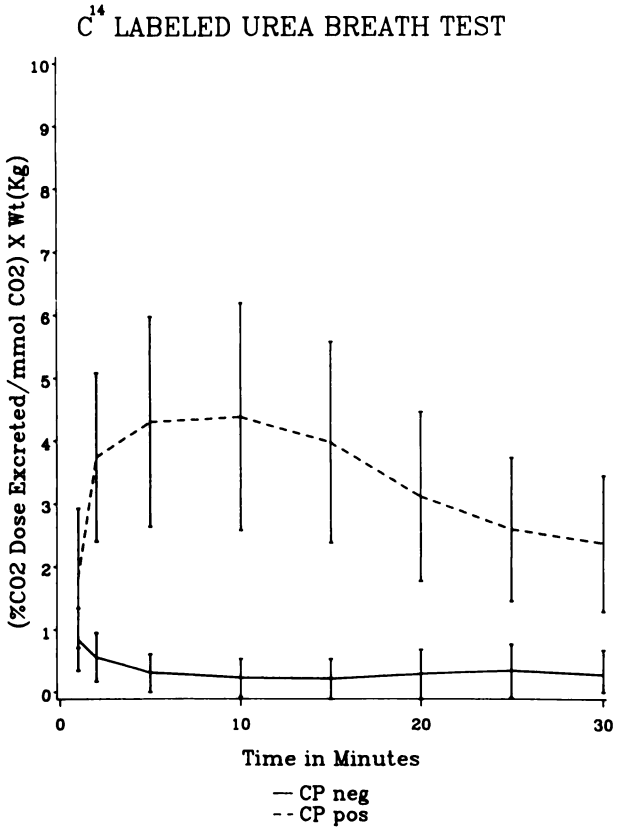

FIGURE 2
Graphed results from the 32 evaluable patients (16 CP+ 16 CP–).

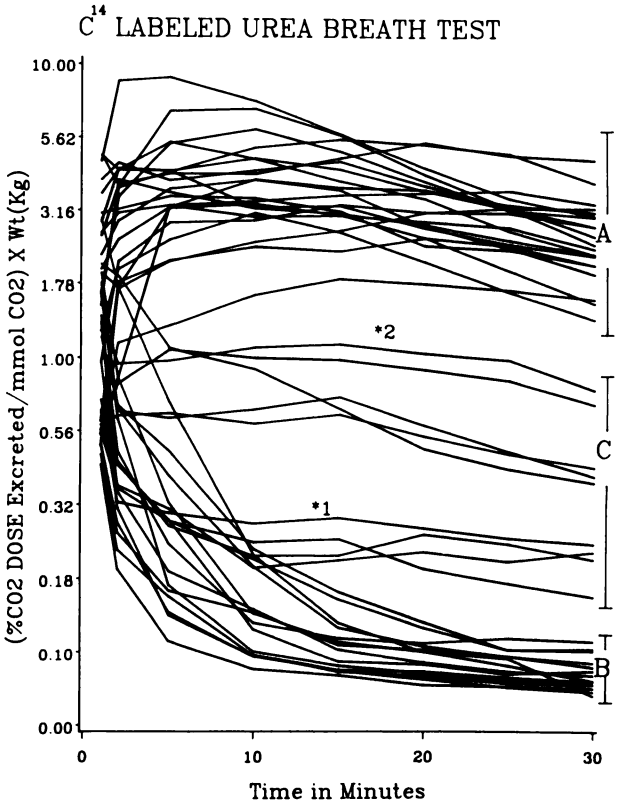

FIGURE 3
Graphed results from 40 "nonevaluable" patients (biopsy not performed or patient on recent antibacterial therapy). There is a high, clearly positive group (A), a low clearly negative group (B), and an equivocal group (C). Patients in group C may have had partial suppression of urease production after antibacterial therapy. *1 is the initial study of a patient who had taken amoxycillin the day before the test. *2 is the same patient 2 wk later when a threefold increase in gastric urease activity had occurred. Note that in this illustration, a logarithmic scale has been used on the "y" axis.

reaction:

urea

$$\begin{array}{l} NH_2 \\ | \\ C{=}O + 2H_2O + H^+ \xrightarrow[urease]{} \end{array} \quad \begin{array}{c} \text{ammonium} \\ 2NH_4^+ \end{array} + \begin{array}{c} \text{bicarbonate} \\ HCO_3^- \end{array}$$

$$\begin{array}{l} | \\ NH_2 \end{array}$$

In the CP+ patients, an average of 40% of the ^{14}C dose was excreted in the urine. The metabolism of the remaining 6 μCi (222 kBq) of ^{14}C can be determined from the paper of Yap et al. (*11*) who studied CO_2 excretion in volunteers who received labeled bicarbonate intravenously. From his data it may be inferred that the radiation dose to body fat for CP+ patients having our test is 44 μGy, bone 180 μGy, lung 6 μGy and gonads 3.6 μGy. In CP– patients 70% of the administered isotope is excreted in the urine so the bladder-wall receives the highest radiation dose. Assuming half the equilibrium dose rate irradiates the bladder wall, the dose to this organ is ~70 μGy (10 μGy = 1mren).

TABLE 3
24-hr Urinary Excretion of ^{14}C as % of Dose Ingested

	No pts.	Mean	s.d.	Min	Max
CP–	11	67	13	47	93
CP+	13	42	12	14	65

DISCUSSION

Serologic studies indicate that ~20% of adults in western countries have colonization of the gastric mucosa with *C. pylori* (*12–15*). In biopsy studies of healthy subjects in Holland (*2*) and the USA (*16*), persons colonized with *C. pylori* all had gastritis, whereas subjects who did not have *C. pylori* had histologically normal gastric mucosa. These studies suggest that evidence of *C. pylori* infection alone is sufficient to make the diagnosis of gastritis.

Gastric urease tests depend on the fact that mammalian cells cannot produce urease (*1*), so the presence of the enzyme always indicates bacterial metabolism. Our test was devised after we observed diminished urea in the gastric juice of *C. pylori* infected patients (*17*) and noted the high specificity of a biopsy urease test for

diagnosing both *C. pylori* infection and active gastritis (*2*). A similar breath test using ^{13}C has been described by Graham et al. (*18*). Graham's test has the advantage of using a nonradioactive isotope. On the other hand, the ^{13}C method is time-consuming and requires a mass spectrometer. Our test is a quick one to perform, so we recall patients with equivocal results for a second test rather than have all patients carry out a prolonged collection. Unlike the results from serologic studies, equivocal breath tests tend to be due to transient aberrations, rather than persistently borderline values of gastric urease activity.

Examination of the 40 patients classified as "nonevaluable" (44 tests) shows that equivocal results occur if the patient has been exposed to antibiotics or bismuth compounds in the weeks preceding the test (Fig. 3). It was our practice to perform endoscopy and biopsy 14 days after antibacterial therapy to test for continuing *C. pylori* infection. The breath test is less sensitive than multiple gastric biopsy specimens, so we recommend a longer period (e.g., 28 days) between cessation of therapy and the test.

Most of the CP+ patients evaluated had duodenal ulcer disease so normal or high acid secretion can be assumed to have been present. We did not test any patients with achlorhydria, or who were actually taking H2-receptor antagonist drugs. When gastric pH rises above 3.0, urease-producing commensal flora from the mouth may grow and give rise to false-positive test results. To avoid this event, drugs such as cimetidine should be stopped 24 hr before the test, and the patient's clinical details and recent medications should be known by the person reporting the test.

This breath test has been a useful additional means of diagnosing *C. pylori* infection at our institution. In other studies (*19*) we have found that serologic tests which detect IgG antibodies to *C. pylori* are sensitive indicators of infection (90% sensitivity for an ELISA test) but cannot be used to confirm eradication of the organism because antibodies decline so slowly after cure. Thus, until now, confirmation of bacteriologic cure required biopsy. It seems wasteful to perform endoscopy merely to obtain a sample of tissue for bacteriologic examination. In most patients, a breath test 28 days after therapy can confirm eradication of the organism. If an equivocal result is obtained, the test may be repeated after a further 14 days.

We hope that the ^{14}C breath test will allow nonendoscopists to study gastritis and *C. pylori* infection. In dyspeptic patients with negative radiologic examinations this test provides useful additional information as to the state of the gastro-duodenal mucosa. If a sufficiently high result is taken as positive for CP (e.g., 1.5 on our scale), false-positive results can be eliminated. The test would then be useful for epidemiologic studies in normals.

When gastric urease activity is high, viable *C. pylori* organisms are almost always present, whereas serologic tests remain positive for many months after eradication of the organism. For this reason, the ^{14}C-urea breath test is the most sensitive and specific noninvasive method presently available for the diagnosis of *C. pylori* infection.

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REFERENCES

1. Delluva AM, Markley K, Davies RE. The absence of gastric urease in germ free animals. *Biochim Biophys Acta* 1968; 151:646–650.
2. Marshall BJ, Francis G, Langton S, et al. Rapid urease test in the management of *Campylobacter pyloridis* associated gastritis. *Am J Gastroenterol* 1987; 3:200–210.
3. Chen XG, Correa P, Offerhaus J, Roderiguex E, Janney F, Hoffmann E, et al.Ultrastructure of the gastric mucosa harboring *Campylobacter*-like organisms. *Am J Clin Pathol* 1986; 86:575–582.
4. Tricottet V, Bruneval P, Vire O, Camilleri JP. *Campylobacter*-like organisms and surface epithelium abnormalities in active, chronic gastritis in humans: an ultrastructural study. *Ultrastructural Pathol* 1986; 10:113–122.
5. McNulty CAM. The treatment of *Campylobacter* associated gastritis. *Am J Gastroenterol* 1987; 82:245–247.
6. Marshall BJ, Warren JR. Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration. *Lancet* 1984; 1:1311–1315.
7. Goodwin CS, Blincow ED, Warren JR, Waters TE, Sanderson CR, Easton L. Evaluation of cultural techniques for isolating *Campylobacter pyloridis* from endoscopic biopsies of gastric mucosa. *J Clin Pathol* 1985; 38:1127–1131.
8. Abt AF, Von Schuching SL. Fat utilization tests in disorders of fat metabolism. A new diagnostic method applied to patients suffering from the malabsorption syndrome, chronic pancreatitis, and atherosclerotic cardiovascular disease. *Bull Johns Hopkins Hosp* 1966; 119:316–330.
9. Kaihara S, Wagner HN. Measurement of intestinal fat absorption with carbon-14 labelled tracers. *J Lab Clin Med* 1968; 71:400–411.
10. Fromm H, Hofman AF. Breath test for altered bile acid metabolism. *Lancet* 1971; 2:621–625.
11. Yap SH, Hafkenscheid JCM, Goossens CMIC, et al. Estimation of radiation dosage and transmutation effect of C-14 involved in measuring rate of albumin synthesis with C14 bicarbonate. *J Nucl Med* 1974; 16:642–648.

12. Eldridge J, Lessels AM, Jones DM. Antibody to spiral organisms in gastric mucosa. *Lancet* 1984; 1:1237.
13. Langenberg ML, Tytgat GNJ, Schipper MEI, et al. *Campylobacter*-like organisms in the stomach of patients and healthy individuals. *Lancet* 1984; 1:1348.
14. Morris A, Nicholson G, Lloyd G, et al. Seroepidemiology of *Campylobacter pyloridis*. *N Z Med J* 1986; 99:657–659.
15. Marshall BJ, McGechie DB, Francis GJ, et al. Pyloric *Campylobacter* serology. *Lancet* 1984; 2:281.
16. Barthel J, Westblom U, Gonzalez F, et al. The predictive value of a simple rapid urease test for C. pyloridis in asymptomatic volunteers. *Am J Gastroenterol* 1986; 81:852.
17. Marshall BJ, Langton SR. Urea hydrolysis in patients with *Campylobacter Pyloridis* infection. *Lancet* 1986; 1:965–966.
18. Graham D, Klein PD, Evans DG, et al. Rapid noninvasive diagnosis of gastric *Campylobacter* by a 13C urea breath test [Abstract]. *Gastroenterology* 1986; 90:1435.
19. Goodwin CS, Blincow E, Peterson G, et al. Enzyme-linked immunosorbent assay for *Campylobacter pyloridis*: Correlation with presence of C. pyloridis in the gastric mucosa. *J. Infect Dis* 1987; 155:488–494.