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$^{15}NH_4^+$ Excretion Test: a New Method for Detection of *Helicobacter pylori* Infection

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A noninvasive test for the detection of *Helicobacter pylori* infection that uses [^{15}N]urea as a tracer has been established. The principle the test is based on is the strong urease activity of *H. pylori*. After oral ingestion, [^{15}N]urea is broken down into ammonia and carbon dioxide by *H. pylori* urease in the stomach. The ammonia is absorbed into the blood and excreted in the urine. The amount of [^{15}N]urea, reflecting the magnitude of *H. pylori* infection, is evaluated by measuring the abundance and excretion rate of ^{15}N in ammonia in the urine. Thirty-six patients were examined in our study. The ^{15}N excretion rates in urine ammonia of patients who were *H. pylori* positive were significantly higher than those of *H. pylori*-negative patients ($P < 0.05$). Twenty-three patients were *H. pylori* positive by Gram stain and culture. The sensitivity of the $^{15}NH_4$ excretion test compared with these techniques was 96%, and no false positives were obtained. The $^{15}NH_4^+$ excretion rates of 13 *H. pylori*-negative subjects were all in the normal range (<0.3%). This method is a simple, precise, highly sensitive, noninvasive, nonradioactive test. It could be used for diagnosis as well as for the followup of patients receiving *H. pylori* eradication therapy, especially children and pregnant women. It could also be used in epidemiological investigation of *H. pylori* infection in a general population.

Helicobacter pylori (formerly *Campylobacter pylori*) is a gram-negative, S-shaped bacterium with strong urease activity (5). Recent studies have shown that *H. pylori* is closely associated with chronic gastritis and peptic ulceration (7). Because of the high prevalence and clinical importance of *H. pylori*, it is important to establish a method which can be widely used for the detection of *H. pylori* infection. Although several detection tests are presently available, most of them are invasive. We report here a new test for the detection of *H. pylori* infection that uses [^{15}N]urea as a tracer. The new method is a simple, precise, sensitive, noninvasive, nonradioactive test. It can be used not only for clinical detection but also for the followup of patients with *H. pylori* infection and for epidemiological studies.

MATERIALS AND METHODS

A total of 36 randomly selected patients (24 males and 12 females; mean age ± standard deviation [SD], 37.8 years ± 13.1) admitted for endoscopic examination in Shanghai Xin Hua Hospital were included in the study. Their medical histories were noted in detail. None of them had taken antibiotics or bismuth during the 2 weeks prior to the study.

Three antral biopsies were taken from each patient by endoscopy. One biopsy sample was used for histological examination, and the other two were used for Gram stain and bacterial culture. The $^{15}NH_4^+$ excretion test was performed the next day.

Bacteria were grown on a selective agar supplemented with 1% soluble starch and 10% defibrinated sheep blood for 3 to 7 days under microaerobic conditions (5% O_2, 10% CO_2, and 85% N_2). Identification was based on typical colony morphology, Gram stain, and positive tests for urease, oxidase, and catalase.

The $^{15}NH_4^+$ excretion test was carried out according to the following protocol. After an overnight fast, the patients were given a test meal (100 ml of 25% glucan), and 15 min later, [^{15}N]urea was administered orally (3 mg of urea per kg of body weight; ^{15}N atom concentration, 56.7%). Urine specimens were collected every 30 min for 2 h. The nitrogen concentration in urine ammonia was measured according to Fern's method (4). Briefly, pure ammonium sulfate was made from the urine ammonia in 10 ml of urine by Comway's dispensing method (2) and pretreated with sodium bromide. The sample was then measured for ^{15}N abundance by using a Finnigan MAT-271 mass spectrometer (instrument conditions: cathode, 5.06 A; emission, 0.04 mA; ion source temperature, 160°C; and precision, 0.1%). The total amount of nitrogen in urine ammonia was determined by Nessler's reagent.

RESULTS

A patient was considered to be infected by *H. pylori* if the Gram stain or the culture or both were positive. The distribution of the cases of *H. pylori* infection according to the endoscopic or histological status is presented in Table 1. The results of the $^{15}NH_4^+$ excretion test showed that the ^{15}N abundance in urine ammonia was markedly higher in patients with *H. pylori* infection than in the absence of this bacterium (Fig. 1).

The ^{15}N excretion rate of 2 h was determined by using the following equation: [(^{15}N in total amount of nitrogen in urine ammonia × % ^{15}N)/amount of ^{15}N in tracer administered orally] × 100. The 2-h excretion rate for 23 *H. pylori*-positive patients was 1.232 ± 0.468% (mean ± SD), and that for 13 *H. pylori*-negative patients was 0.169 ± 0.068% ($P < 0.05$, *t* test). The mean ± 2 SD of ^{15}N excretion for 23 *H. pylori*-positive patients was within the range of 0.296 to 2.172%, and that for the 13 *H. pylori*-negative patients was 0.037 to 0.301%. Three percent (mean + 2 SD of negative values) was considered the cutoff value. The correlation between the

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TABLE 1. Distribution of *H. pylori* infection in 36 patients according to their endoscopic or histological status

H. pylori status	No. (%) of patients with[a]:					
	NUD		GU	DU	CU	Bleeding in UDT
	CSG	CAG				
Positive	3 (30)	8 (67)	8 (80)	2 (100)	1 (100)	0 (0)
Negative	7 (70)	4 (33)	2 (20)	0 (0)	0 (0)	1 (100)

[a] NUD, nonulcer dyspepsia; CSG, chronic superficial gastritis; CAG, chronic atropic gastritis; GU, gastric ulceration; DU, duodenal ulceration; CU, complex ulceration; UDT, upper digestive tract.

$^{15}NH_4^+$ excretion test and microbiological tests is presented in Table 2.

In order to test the reproducibility of the method, three patients who were positive for *H. pylori* and three patients who were negative and had not taken any drugs since the first examination were tested with [^{15}N]urea a second time 2 weeks later. The results are shown in Fig. 2. Although there is some variation between the results of the two tests, the results remained within the range of our diagnostic limit.

Four healthy volunteers (two males, two females) with no recent abdominal complaint or disease of the kidney, liver, or endocrine system were tested in order to learn whether the new test could be used for epidemiological investigation in populations. Their ^{15}N excretion rates were 0.098, 0.149, 0.174, and 0.1%.

Moreover, the $^{15}NH_4^+$ excretion test could be a good way to monitor medical treatment of *H. pylori* infection. Three patients (two with ulceration, one with type B gastritis) with *H. pylori* infection and complaints such as stomach pain and occult blood in stools were treated with bismuth salt (subcitrate, 110 mg four times a day). After a 2-week treatment, they were tested a second time by the new test. The ^{15}N excretion rates were all below the cutoff level of 0.3% (Fig. 3). At the same time, their symptoms disappeared. An endoscopy was subsequently performed on one of the three, who was found to be negative for *H. pylori*.

TABLE 2. Correlation between $^{15}NH_4^+$ excretion test and microbiological examination in detecting *H. pylori* infection

Type of test	No. of patients[a]			
	G^+C^+	G^+C^-	G^-C^+	G^-C^-
Microbiological (n = 36)	16	5	2	13
Positive $^{15}NH_4^+$ excretion (n = 36)	16	4	2	0

[a] G, Gram stain; C, bacterial culture.

DISCUSSION

Since the discovery of *H. pylori* and its association with gastritis and peptic ulceration, many tests have been proposed for the detection of this infection. These include tests performed on biopsy samples (urease tests, histological and smear examinations, and culture), noninvasive tests, [^{13}C]urea or [^{14}C]urea breath tests, and serological tests for specific antibody detection. However, all these tests have limits. Therefore, no test is an ideal test for diagnosis, monitoring of treatment, or epidemiological investigation.

The $^{15}NH_4^+$ excretion test is a simple, reproducible, highly sensitive, noninvasive method for the detection of *H. pylori* infection. Patients are required only to have a test meal and then to collect urine samples during a 2-h period.

The $^{15}NH_4^+$ excretion test is a global test reflecting the overall situation of *H. pylori* infection in the stomach, similar to the [^{13}C]urea or [^{14}C]urea breath tests (11). However, the new test has the advantage over the [^{14}C]urea breath test (1, 9, 14, 15, 17) of not using a radioactive element. Although the radioactivity of ^{14}C is not strong, its half-life is up to 5,730 years. Therefore, it cannot be widely used in any population and is absolutely prohibited in children and pregnant women (9). In comparison with ^{13}C (3, 6), ^{15}N and ^{13}C are both stable isotopes, but it is easier to measure [^{15}N]urea than [^{13}C]urea because the natural abundance of ^{15}N (0.00365%) is lower than that of ^{13}C (0.111%). It is easier to label ^{15}N than ^{13}C, which makes the cost of our test much less than that of the [^{13}C]urea breath test (1). Furthermore, it

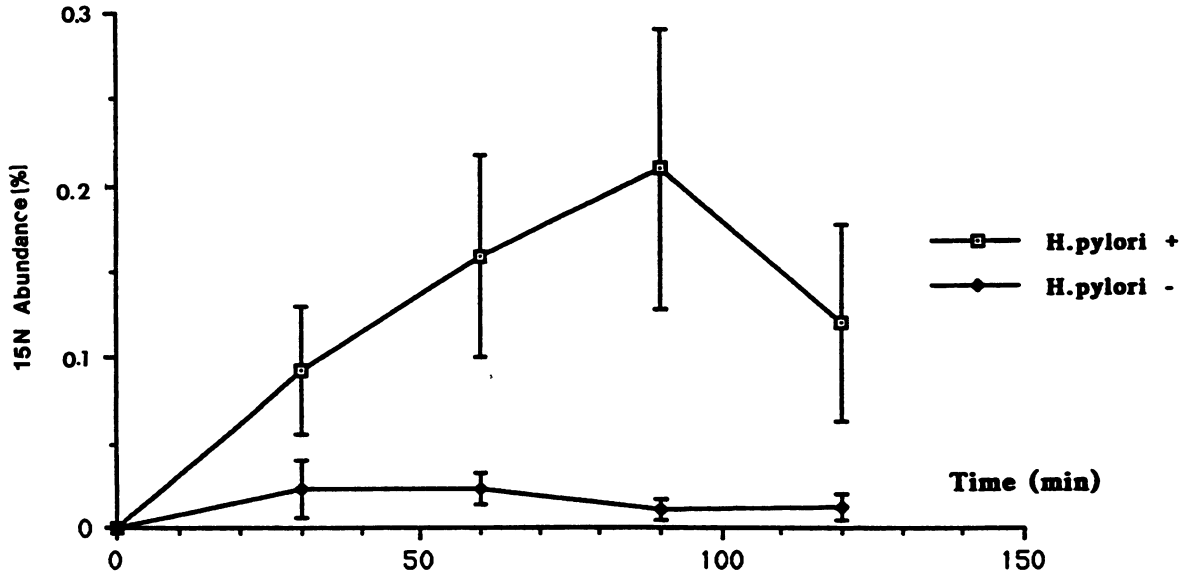

FIG. 1. Abundance of $^{15}NH_4^+$ in urine after ingestion of [^{15}N]urea.

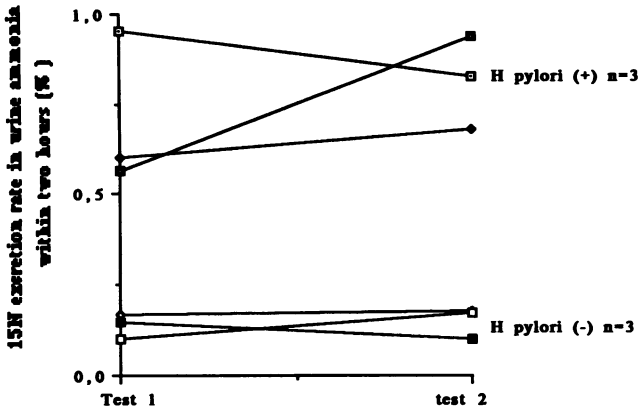

FIG. 2. Reproducibility of $^{15}NH_4^+$ excretion test with *H. pylori*-positive and -negative patients.

is also easier to measure the ^{15}N excretion rate in urine than the ^{13}C excretion rate in breath samples. A limit to this test, as for the breath tests, is the eventual false-positive results obtained if *Gastrospirillum hominis* (10, 13) is present, but this is seldom encountered.

Although serological testing for specific *H. pylori* antibodies is also a simple method (8, 12) which, to a certain extent, reflects the magnitude of *H. pylori* infection in the stomach, the serum antibody levels are maintained for a long time after *H. pylori* is eradicated. Therefore, such testing cannot be used as a followup technique in the treatment of *H. pylori* infection (16). In contrast, the $^{15}NH_4^+$ excretion test, like the breath tests, theoretically shows the actual state of *H. pylori* infection in the stomach. Although the data presented in this paper concerning monitoring of treatment are preliminary, we are confident that this test will be helpful in the future.

The following precautions are necessary when the $^{15}NH_4^+$ excretion test is performed.

(i) An overnight fast prior to the test is required because meal consumption dilutes the concentration of urease in the stomach, particularly if the infection is weak, and thus can produce a false-negative result. In our experience, one patient who had an *H. pylori* infection detected by Gram stain was negative by the [^{15}N]urea test because he drank two bottles of milk before the test. The purpose of using 25% glucan as a test meal is to slow the emptying of the stomach and thus increase the time of interaction between [^{15}N]urea and *H. pylori*.

(ii) It is necessary to rinse the mouth before the test to avoid contamination by normal mouth flora.

(iii) It is important to collect urine for 2 h, because the excretion of ^{15}N in urine ammonia peaks within 1 to 2 h after the tracer is ingested. Several drops of HCl should be added to prevent evaporation of urine ammonia. The urine can be stored at −30°C for 2 weeks without any obvious change in ^{15}N abundance in urine ammonia.

In verifying the reproducibility of our test, two *H. pylori*-positive and three *H. pylori*-negative patients were retested by the $^{15}NH_4^+$ excretion test. The results were consistent with the former diagnosis, although there was a slight fluctuation in the amount, which may have been related to the interference of blood ammonia coming from the intestines or to a change in blood pH.

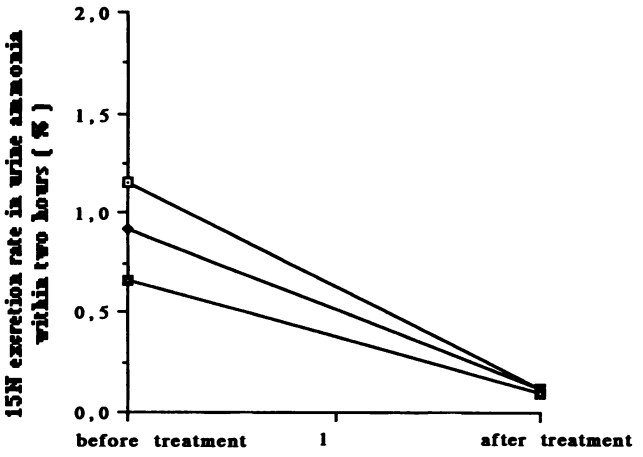

FIG. 3. Effects in three patients of eradicating *H. pylori*. The ^{15}N excretion test was used.

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REFERENCES

1. **Bell, G. D., J. Weil, G. Harrison, et al.** 1987. ^{14}C-urea breath analysis, a non-invasive test for *Campylobacter pylori* in the stomach. Lancet **i**:1367–1368.
2. **Birkhahn, R. H., C. L. Long, D. Fitkin, M. Joevanandam, and W. S. Blackmore.** 1987. Whole body protein metabolism due to trauma in man as estimated by L-(^{15}N)-alanine. Am. J. Physiol. **241**:E64.
3. **Dill, S., J. J. Payne-James, Misiewicz, G. K. Grimble, D. McSwiggan, K. Pathak, A. J. Wood, C. M. Scrimgeons, and M. J. Rennie.** 1990. Evaluation of ^{13}C urea breath test in the detection of *Helicobacter pylori* and in monitoring the effect of tripotassium dicitratobismuthate in non ulcer dyspepsia. Gut **31**:1237–1241.
4. **Fern, E. B., et al.** 1981. The excretion of isotope in urea and ammonia for estimating protein turnover in man with ^{15}N glycine. Clin. Sci. **61**:217–228.
5. **Goodwin, C. S., J. A. Armstrong, T. Chilvers, M. Peters, M. D. Collins, L. Sly, W. McConnell, and W. E. S. Harper.** 1989. Transfer of *Campylobacter pylori* and *Campylobacter mustelae* to *Helicobacter* gen. nov. as *Helicobacter pylori* comb. nov. and *Helicobacter mustelae* comb. nov., respectively. Int. J. Syst. Bacteriol. **39**:397–405.
6. **Graham, D. Y., P. D. Klein, D. J. Evans, Jr., et al.** 1987. *Campylobacter pylori* detected noninvasively by the ^{13}C-urea breath test. Lancet **i**:1174–1177.
7. **Jiang, S. L., W. Z. Liu, D. Z. Zhang, et al.** 1987. Campylobacter-like organisms in chronic gastritis, peptic ulcer, and gastric carcinoma. Scand. J. Gastroenterol. **22**:553–558.
8. **Kosunen, T. U., J. Höök, H. I. Rautelin, and G. Myllylä.** 1989. Age-dependent increase of *Campylobacter pylori* antibodies in blood donors. Scand. J. Gastroenterol. **24**:110–114.
9. **Marshall, B. J., and I. Surveyor.** 1988. Carbon-14 urea breath test for the diagnosis of *Campylobacter pylori* associated gastritis. Nucl. Med. **29**:11–16.
10. **McNulty, C. A. M., J. C. Dent, A. Curry, J. S. Uff, G. A. Ford, M. W. L. Gera, and S. P. Wilkinson.** 1989. New spiral bacterium in gastric mucosa. J. Clin. Pathol. **42**:585–591.
11. **Mégraud, F.** 1988. Comparison of different tests for *Campylobacter pylori*. Scand. J. Gastroenterol. **23**(Suppl. 142):64–68.
12. **Mégraud, F., M.-P. Brassens-Rabbé, F. Denis, A. Belbouri, and D. Q. Hoa.** 1989. Seroepidemiology of *Campylobacter pylori* infection in various populations. J. Clin. Microbiol. **27**:1870–1873.
13. **Morris, A., M. R. Ali, L. Thomsen, and M. Vanderwee.** 1989. Tightly spiral shaped bacteria in gastric antrum: are they urease

positive? J. Clin. Pathol. **42**:216.

14. **Ormand, J. E., N. J. Talley, H. A. Carpenter, R. G. Shorter, C. R. Conley, W. R. Wilson, E. P. DiMagno, A. R. Zinsmeister, and S. F. Phillips.** 1990. ^{14}C urea breath test for diagnosis of *Helicobacter pylori*. Dig. Dis. Sci. **35**:879–884.
15. **Rauws, E. A. J., E. A. V. Royen, W. Langenberg, J. V. Woensel, A. A. Vrijj, and G. N. Tytgat.** 1989. ^{14}C-urea breath test in *Campylobacter pylori* gastritis. Gut **30**:798–803.
16. **Vaira, D., and J. Holton.** 1989. Serum immunoglobulin G antibody levels for *Campylobacter pylori* diagnosis. Gastroenterology **97**:1069.
17. **Veldhuyzen Van Zanten, S. J. O., K. M. A. J. Tytgat, J. Hollingsworth, S. Jalali, F. A. Rashid, B. M. Bowen, J. Goldie, R. L. Goodacre, R. H. Riddell, and R. H. Hunt.** 1990. ^{14}C-urea breath test for the detection of *Helicobacter pylori*. Am. J. Gastroenterol. **85**:399–403.