METHODS

A Method for Estimation of Urease Activity in Gastric Mucosa Biopsy Specimens and *Helicobacter pylori* Cell Suspensions

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A method for measuring urease activity in biopsy specimens and *Helicobacter pylori* cultures from these specimens is proposed. The method is based on measurement (with a portable pH-meter) of the rate of pH changes in a reaction mixture consisting of buffer, substrate (urea), and biopsy specimen or bacterial cells. This method revealed that urease activity of biopsy specimens correlated with that of *H. pylori* suspension in the same experiment. High urease activity was found in biopsy specimens containing the greatest number of *Helicobacter* cells; only one of 14 specimens free of *H. pylori* cells showed no urease activity. Introduction of this method into clinical practice will help to evaluate the contribution of *H. pylori* to the pathological process.

Key Words: *Helicobacter pylori; biopsy specimens; urease activity*

Helicobacter pylori are gram-negative microaerophilic bacilli inhabiting human gastric mucosa and often participating in ulcer formation [1].

A characteristics feature of *H. pylori* is the synthesis of urease (urea amidohydrolase, EC 3.5.1.1). Urease produced by *H. pylori* catalyzes urea hydrolysis with the formation of ammonium and hydroxyl ions. Urease is one of actively studied *H. pylori* proteins. It is essential for colonization of gastric mucosa by these bacteria. This enzyme is currently used for taxonomic identification of *H. pylori*, it is one of the main *H. pylori* antigens and a candidate for vaccine development [5]. Urease activity in patients with peptic ulcer is believed to augment the pathological process. Urease activity (UA) in biopsy specimens, along with other tests, usually indicates the presence of *H. pylori* in the stomach. Our aim was to develop a rapid quantitative method for measuring UA in biopsy specimens. This can help to determine the degree of gastric mucosa colonization with *H. pylori* and to elucidate the role of this bacterium in ulcer formation.

MATERIALS AND METHODS

We examined 20 female (age 31-69 years) and 4 male patients (age 37-69 years) with peptic ulcers. Biopsy specimens of the gastric mucosa were collected from the pylorus. The presence of *H. pylori* was detected using the following tests (considered now in clinical practice as gold standard): microscopic examination of Gram-stained impression smears, qualitative detection of UA in biopsy ma-

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terial, and isolation of cultures from biopsy specimens (inoculation into blood agar) [1]. There are three basic methods for UA measurement: colorimetry (measurement of ammonium release using Nessler reagent) [6], titrimetry (by the amount of hydrochloric acid needed for ammonium neutralization) [3], and pH measurement (pH changes as a result of ammonium release during urea hydrolysis in reaction mixture without buffer) [5].

We selected and modified the third of these methods. Freshly obtained biopsy specimens were transported into laboratory in sterile saline (pH 6.9-7.9). Normal saline for reaction mixture was prepared directly before measurements. Biopsy specimen was put into a tube with 1.5 ml saline without buffer and the electrode of Piccolo-2 pH-meter (Hanna), 12 mm in diameter, was plunged in the mixture. After 10-15-min incubation at 37°C, pH was measured twice with 10-min interval without removing the tube from the thermostat and at constant stirring of the reaction mixture. The initial pH was 6.8-7.2. Then 80 µl urea was added (final concentration 600 µg/ml) and pH was measured immediately after substrate addition and every 10 min for 30-50 min. Figure 1, *a*, presents three typical curves of pH dynamics, reflecting UA of biopsy specimens. UA was calculated as the initial linear rate of pH changes: pH difference between the start and end of a straight fragment standardized by the weight of biopsy specimen and the time of incubation. UA value was expressed in pH units/h/mg biopsy specimen.

H. pylori cultures were grown in blood agar with antibiotics in microaerobic atmosphere with 84% N_2, 10% CO_2, and 6% O_2. The dishes were incubated at 37°C for 3-7 days [7]. *H. pylori* cultures presented as round smooth, glossy semitransparent colonies with even edges, 0.3-1.5 mm in diameter. The cultures were identified by qualitative tests for urease, catalase, and oxidase activity [2]. Characteristic gram-negative spiral and curved *H. pylori* cells were detected in Gram-stained smears.

Urease activity of 3-7-day cultures of *H. pylori* grown in blood agar was measured in cell suspension washed twice in normal saline. An aliquot of *H. pylori* cell suspension, containing 2-15 µg protein in 5-20 µl, and urea were added into the tube with 1.5 ml unbuffered saline (as described above). In order to obtain reliable data, the volume of suspension was selected so that the initial linear fragment of the pH-time curve passed through 4-5 pH points measured with 1-min intervals. After selecting the optimal volume of the aliquot, at least 3 measurements were carried out, and the mean UA was estimated. UA was expressed in pH units/min/mg protein. Figure 1, *b*, shows typical curves of pH time course, reflecting UA of *H. pylori* cultures.

Protein was measured by the method of Lowry. Pierson's linear correlation coefficient was calculated using Microsoft Excel software.

RESULTS

UA was detected in all biopsy specimens except one; impression smear of this specimen contained no *H. pylori* cells (Table 1). Other specimens contained bacteria in different quantities.

Growth in blood agar did not coincide with the presence of cells in the specimen. No growth was observed in specimens Nos. 3, 4, 8, 11, and 13, though they contained *H. pylori* cells (Table 1).

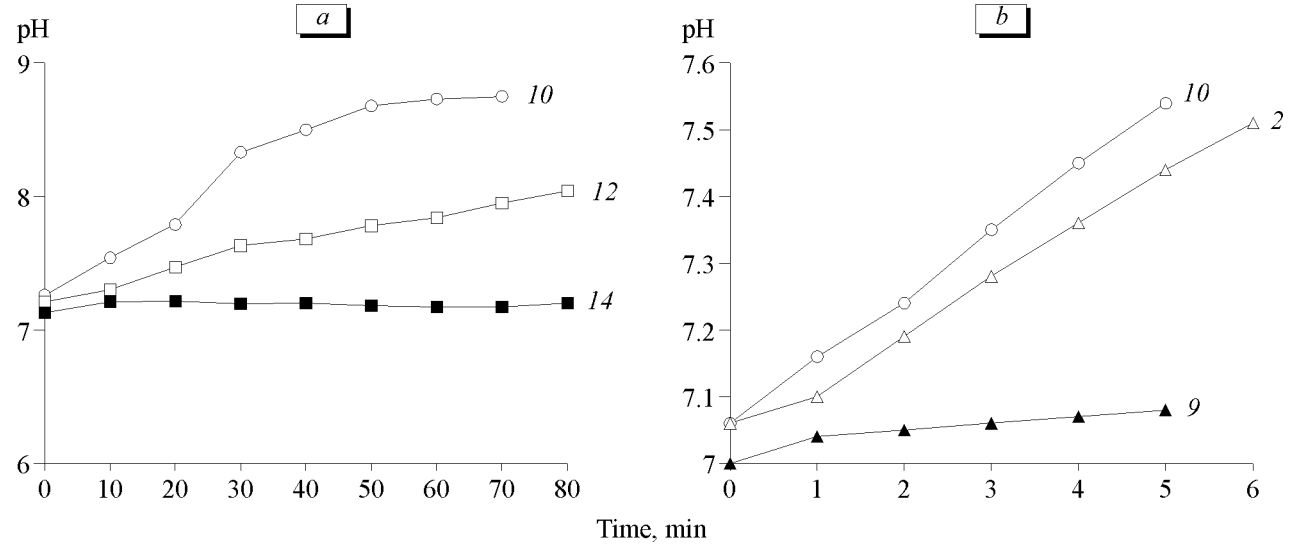

Fig. 1. Urease activity of biopsy specimens (*a*) and *H. pylori* cultures (*b*). Italic figures near the curves show the number of biopsy specimen or culture.

TABLE 1. Study of Biopsy Specimens Isolated from the Pyloric Part of the Stomach from Patients with Peptic Ulcer

Specimen No. (patient's age, sex)	Presence of *H. pylori* cells in smear impression	Growth of *H. pylori* in blood agar	Weight of specimen, mg	UA, pH units/h/mg
1 (66; F)	++	+	1.2	0.121
2 (63; F)	+++	+	4.2	0.875
3 (48; F)	+++	—	13.2	0.129
4 (51; F)	+	—	2.0	2.520
5 (31; F)	++	+	3.0	0.293
6 (50; M)	+	+	2.5	0.108
7 (69; F)	++	+	5.2	0.531
8 (63; F)	++	-	4.0	0.098
9 (63; F)	++	+	2.4	0.086
10 (32; F)	++++	+	0.8	2.675
11 (58; M)	++	—	4.4	0.500
12 (52; F)	+++	+	5.8	0.120
13 (37; M)	+++	—	6.0	0.170
14 (69; M)	—	—	6.0	0

Note. Presence of *H. pylori* in a smear impression: "-" none; "+" several cells in the microscope visual field; "++" 10-20 cells in a visual field; "+++" 20-50 cells; "++++" more than 50 cells in a visual field; growth in blood agar: "+" present; "-" absent.

The maximum content of bacterial cells in the specimen corresponded to the maximum UA (Table 1, specimen No. 10), while the absence of cells in the impression smear (No. 14) was confirmed by the absence of UA and growth in blood agar.

UA was repeatedly measured in all cultures. Different results can be explained by different growth phases. In one experiment we compared UA of cultures Nos. 2, 9, and 10 (18.47±2.04, 2.5± 0.3, and 28.07±3.02 pH units/min/mg protein, respectively) with UA of the respective biopsy specimens (0.875, 0.086, and 2.676 pH units/h/mg specimen) and revealed a linear correlation (r=0.934) between these parameters. These results suggest that the higher is UA of *H. pylori* culture, the higher UA in the biopsy specimen.

Since urease is one of the factors of *H. pylori* pathogenicity, augmenting the inflammatory process in the stomach [4], the use of quantitative parameters of UA in biopsy specimens will help to evaluate the contribution of *H. pylori* to the pathological process. Moreover, our method for measuring UA activity in cultures will be useful in further investigation of physiological and biochemical characteristics of *H. pylori*.

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