

Measurement of urea and ammonium concentrations in gastric juice

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Abstract

Aim—To study the effect of known interference in the measurement of urea and ammonium concentrations in samples of gastric juice.

Method—The effect of pH and ammonium concentration on the o-phthalaldehyde method, the diacetylmonoxime method, a Berthelot linked method and an enzymatic urease method for the measurement of urea in gastric juice was therefore conducted. An enzymatic method of the measurement of ammonium in gastric juice was also assessed.

Results—The o-phthalaldehyde and the enzymatic urease methods were unaffected by a low gastric juice pH, ammonium concentrations of 10 mmol/l, and had interassay coefficients of variation of 3.9–5.6% and 2.8–10.6%, respectively, over a urea concentration of 2.5 mmol/l–20 mmol/l. The Berthelot linked method resulted in low gastric juice urea concentrations. The enzymatic method of ammonium measurement also proved suitable when the effect of low gastric juice pH was controlled.

Conclusion—Interference by low pH did not explain the differences in reports of gastric juice urea or ammonium concentrations.

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There is a clear association between the development of duodenal ulcer disease and infection of the gastric antral mucosa by *Helicobacter pylori*.^{1,2} This micro-organism has abundant urease activity which enables it to hydrolyse gastric juice urea with the production of ammonium and carbon dioxide.^{3,4} Its urease activity has been used to detect the presence of infection by the ¹⁴C-urea breath test and by the rapid urease test (CLO test) after antral biopsy.^{5,6} Samples of gastric juice for the measurement of urea and ammonia concentration can be readily obtained during diagnostic upper gastrointestinal endoscopy or by nasogastric intubation. As a result it has also been suggested that the measurement of the gastric juice concentrations of either urea or ammonium alone may also be used to detect this infection. Workers disagree about which of these two gastric juice constituents best detects the presence of *H pylori* infection. Marshall *et al* considered that gastric juice urea concentration best detected infection and reported mean concentrations of 0.45 mmol/l in subjects with *H pylori* infection and

2.9 mmol/l in those not infected.⁷ Others have found gastric juice urea concentrations of 3.27 mmol/l and 4.8 mmol/l in infected subjects and no difference between infected and non-infected subjects.^{8,9}

Marshall *et al* reported mean ammonium concentrations of 34 mmol/l and 11 mmol/l in infected and non-infected subjects, respectively. Kim *et al* found that gastric juice ammonium concentrations were a good predictor of infection if a threshold of 3 mmol/l was exceeded, and reported mean concentrations of 5.48 mmol/l and 1.26 mmol/l, respectively.⁸

The methods used to measure urea and ammonium concentrations in previous reports have not been well described. As a low gastric juice pH and the presence of ammonium ions could interfere in the measurement of urea and a low pH could also interfere in the enzymatic measurement of ammonium these different reports may be explained by the use of different methods of analysis. An assessment of methods of measuring urea and ammonium concentrations in gastric juice samples was therefore conducted.

Methods

Following the passage of a nasogastric tube and pentagastrin stimulation gastric juice was collected from one healthy volunteer who had *H pylori* infection. The gastric juice sample was stored at -20°C until analysis.

The effects of pH and gastric juice ammonium concentration on the imprecision, inaccuracy, and detection limit of four methods of urea analysis were investigated. These were a rate reaction enzymatic method automated on the Cobas Bio (Roche, Welwyn Garden City, Herts, England), the o-phthalaldehyde method automated on the Excel analyser (American Monitor, West Sussex, England), a manual diacetylmonoxime method (Sigma Chemical Company, Dorset, England) and a manual urease method linked to the Berthelot reaction (Sigma Chemical Company, Dorset, England). The effect of pH on the inaccuracy and imprecision of the enzymatic ammonia method automated on the Cobas Bio (Roche, Welwyn Garden City, Herts, England) (Sigma Chemical Company, Dorset, England) was also investigated.

EFFECT OF pH ON THE MEASURED GASTRIC JUICE UREA CONCENTRATION

After measuring the basal pH of the gastric juice using a Corning 220 pH meter (Corning, Herts, England) 75 µl of 2 mol/l

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Table 1 Effect of gastric juice pH on measurement of urea concentrations after addition of urea, to give 5 mmol/l concentration

| | Mean urea concentration in gastric juice (mmol/l) | | | |
|--------------------------------|---|---------|---------|---------|
| | pH 1.56 | pH 4.13 | pH 6.49 | pH 1.60 |
| o-Pthalaldehyde method | 4.8 | 4.6 | 4.6 | 4.9 |
| Diacetylmonoxime method | 4.5 | 4.3 | 4.3 | 4.9 |
| Urease method | | | | |
| unblanked | 7.0 | 6.9 | 6.9 | 6.6 |
| (blanked) | (5.2) | (5.1) | (5.2) | (4.8) |
| Berthelot linked urease method | 4.3 | 3.0 | 3.0 | 4.0 |

sodium hydroxide was added to a 5 ml portion. This increased the pH to 4.0 and a portion of this gastric juice was collected. The pH of the remainder was again increased to pH 6.5 by the addition of more sodium hydroxide solution and a portion was again collected. The pH of the remaining gastric juice was then titrated back to the original value by the addition of about 50 μ l of 1 mol/l hydrochloric acid to exclude any dilutional effect on the measured urea concentration. Portions (0.9 ml) of the four pH adjusted gastric juice samples then had 0.1 ml of stock 50 mmol/l urea solution or deionised ammonia free water added to produce gastric juice samples with a low urea concentration, or 5 mmol/l urea.

The inaccuracy of the measurement of urea in gastric juice samples with their native unadjusted pH was assessed by calculating the recovery of urea in portions of gastric juice to which urea had been added to give concentrations of 2.5, 5, 10 and 20 mmol/l. The samples were assayed by each method in triplicate.

EFFECT OF AMMONIUM CHLORIDE ON MEASURED UREA CONCENTRATION

Gastric juice samples had 0.1 ml of 100 mmol/l solution of ammonium chloride added to increase the total ammonium concentration by 10 mmol/l. These samples containing ammonia were split into two portions and stock urea solution added to give a final urea concentration of 5 mmol/l in one portion. The concentrations of urea were then measured in each of the portions of gastric juice by all four methods.

EFFECT OF pH ON THE MEASURED GASTRIC JUICE AMMONIUM CONCENTRATION

Samples of gastric juice had their pH adjusted as described for urea. Portions of 100 mmol/l ammonium chloride solution were then added to produce 5 mmol/l and 10 mmol/l ammonia concentrations. Samples were then analysed in quintuplicate for ammonium concentration using an enzymatic method

(Sigma, Dorset, England) adapted for the Cobas Bio centrifugal analyser.

The inaccuracy of the ammonia method was assessed by measuring the recovery of ammonium chloride which was added to portions of gastric juice to give final concentrations of 2.5, 5, 7.5 and 10 mmol/l. Basal gastric juice samples collected from several other subjects who had *H. pylori* infection were analysed in replicate to assess the imprecision of measurement using native gastric juice.

Results

EFFECT OF pH ON UREA MEASUREMENT

A change in the pH of the samples did not noticeably interfere with the measurement of urea by the o-pthalaldehyde, diacetylmonoxime, or the blanked urease methods (table 1). The Berthelot linked urease method resulted in lower concentrations of urea at each pH tested. The use of 0.2 mol/l phosphate buffer as a diluent for the Berthelot method did not increase the urea concentrations obtained by this method. The enzymatic urease method required blanking as the mean basal gastric juice urea concentration was 1.8 mmol/l with this method compared with 0.2 mmol/l using the other methods.

The mean (range) percentage recovery of added 5 mmol/l urea with the blanked enzymatic urease method was 102% (97–105%), with the o-pthalaldehyde method 95% (92–98%), with the diacetylmonoxime method it was 90% (89–98%), and with the Berthelot reaction it was 72% (60–86%).

The interassay coefficient of variation (CV) of analysis at each of the urea concentrations investigated showed that the blanked urease enzymatic method and o-pthalaldehyde methods were least imprecise (table 2).

EFFECT OF AMMONIUM ON THE MEASUREMENT OF UREA

The addition of 10 mmol/l ammonium chloride to the gastric juice did not interfere with the measurement of urea by either the diacetylmonoxime method or the o-pthalaldehyde method. The addition of ammonium chloride resulted in the detection of a urea concentration of 4.2 mmol/l by the enzymatic urease method compared with a mean of 0.96 mmol/l by the other methods. When a blank sample was also analysed then interference by ammonium was controlled.

EFFECT OF pH ON THE MEASUREMENT OF GASTRIC JUICE AMMONIUM

A change in gastric juice pH over the range 2.29 to 7.20 did not affect the measurement of ammonium (table 3). The samples were diluted 1 in 4 or 1 in 20 (before analysis) depending on the ammonium concentration with 0.2 mol/l phosphate buffer (pH 7.4). This reduced the concentration of ammonia in the sample to that of the linear range of the assay which was between 30 μ mol/l and 1 mmol/l. The phosphate buffer neutralised the effects of gastric juice acidity. If distilled

Table 2 Interassay imprecision in measurement of urea in gastric juice samples

| | Coefficient of variation at a urea concentration of: | | | |
|------------------------------------|--|----------|-----------|-----------|
| | 2.5 mmol/l | 5 mmol/l | 10 mmol/l | 20 mmol/l |
| o-Pthalaldehyde method (%) | 5.4 | 6.0 | 4.6 | 3.9 |
| Diacetylmonoxime method (%) | 16.0 | 12.0 | 9.8 | 8.3 |
| Urease method blanked (%) | 5.4 | 2.8 | 10.2 | 10.6 |
| Berthelot linked urease method (%) | 45.6 | 20.4 | 33.5 | 59.2 |

Table 3 Absence of any effect of pH of gastric juice samples on measurement of ammonium concentration ($n = 5$) using enzymatic method adopted for Cobas Bio

| | Gastric juice ammonium concentration (SD) at a range of sample pH: | | | |
|-----------------------------------|--|-----------------|-----------------|-----------------|
| | pH 2.29 | pH 4.04 | pH 7.20 | pH 7.29 |
| Ammonium chloride not added | 0.98 (0.010) | 0.97 (0.016) | 0.93 (0.016) | 0.90 (0.016) |
| 5 mmol/l added ammonium chloride | 5.5 (0.14) | 5.0 (0.05) | 5.4 (0.07) | 5.2 (0.26) |
| 10 mmol/l added ammonium chloride | 9.8 (0.16) | 9.8 (0.07) | 9.8 (0.51) | 9.4 (0.26) |

water was used as the diluent then there was an apparent reduction in measured concentrations of ammonia.

The intra-assay coefficient of variation of the standard with 10 mmol/l ammonium concentration was 1.0% while the interassay coefficient of variation was 2.0%. Using samples from patients the intra-assay coefficient of variation including dilution varied from 8.5% at an ammonium concentration of 2.3 mmol/l to 1% at an ammonium concentration of 13.0 mmol/l.

The interassay coefficient of variation of the enzymatic ammonia method (including dilution) using the gastric juice samples with added ammonium chloride ranged from 17.2% at an ammonium concentration of 2.5 mmol/l to 6.5% at an ammonium concentration of 7.5 mmol/l. The interassay coefficient of variation from a range of samples from several subjects ranged from 8.5% at 2.3 mmol/l to 1.5% at an ammonium concentration of 11.1 mmol/l.

The measured ammonium concentration in the samples was linear with serial dilution. The detection limit of the assay was 30 μ mol/l.

Discussion

This study has shown that the differing reports of urea concentration in gastric juice samples⁷⁻⁹ cannot be explained by the effect of gastric juice pH on different methods of urea analysis. It has also shown that the enzymatic urease method, when used without blanking, resulted in an apparent increase in urea concentration due to the presence of high concentrations of ammonium which may occur with infection by *H pylori*. In the two studies which found no difference in the urea concentration in gastric juice before and after eradication of *H pylori* urease methods were used.^{8,9} If these methods had not been blanked then this might explain the results.

Raised plasma urea concentrations result in an increased concentration of urea in gastric juice. We have already shown that the production of ammonia by *H pylori* urease activity may be stimulated by the infusion of urea.¹⁰ Raised plasma urea concentration in subjects with chronic renal failure therefore results in raised gastric juice urea concentrations and raised gastric juice ammonium concentrations.⁸ The reports of high concentrations of gastric juice urea in subjects with *H pylori* infection could therefore also be explained by raised plasma urea concentrations.

The o-phthalaldehyde method and the enzymatic urease method, when blanked, proved the most suitable methods for the measurement of urea. The Berthelot method of measurement of urea did not prove suitable for use with gastric juice samples. Its imprecision and inaccuracy, when compared with the other manual method, suggested an interference in the method. This was not due to the pH of the sample because buffering the sample pH by reconstituting the urease enzyme with 0.2 mol/l phosphate buffer (pH 7.4) instead of the deionised water did not eliminate the interference. It may have been due to a matrix effect of the gastric juice, such as the concentration of bile acids. Although this method could account for apparent differences in gastric juice urea or ammonium concentrations, it was not used in any of the three studies reported.⁷⁻⁹

This study has also shown that the differing reports of gastric juice ammonium concentration might be explained by the effect of gastric juice pH. The pH of the sample did not interfere in the measurement of ammonium concentration when the samples were diluted with 0.2 mol/l phosphate buffer (pH 7.4) before analysis to bring the ammonia concentration to within the linear range of the method. If the gastric juice acidity had not been neutralised by the diluent buffer then this caused an apparent reduction in gastric juice ammonium concentrations. This might account for reports of low gastric juice ammonium concentrations in the presence of *H pylori* infection.

The differences in reports of gastric juice urea and ammonium concentrations might also have been due to a failure to distinguish properly between those subjects with *H pylori* infection and those without.

In conclusion, the enzymatic urease method for the measurement of urea and the o-phthalaldehyde method proved the most suitable for the measurement of urea in gastric juice samples.

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