The causal relationship between Helicobacter pylori colonization of the gastric mucosa and gastritis is well accepted. Endoscopy and subsequent histological examination of antral biopsies have been regarded as the gold standard for diagnosing H pylori gastritis. The 13C urea breath test (UBT) is an attractive diagnostic alternative that offers numerous advantages. It is highly reliable, safe, nonradioactive, noninvasive and independent of expired air volumes.

The 13C urea breath test is based on the production of urease by the organism. In 1954, Kornberg et al (1) showed that antibiotics destroy gastric urease in cats and thereby concluded that urease was bacterial in origin. In 1983, War-...
Comparison of $^{13}$C urea breath test validation studies in children and adolescents

<table>
<thead>
<tr>
<th>Authors (reference)</th>
<th>Sensitivity/ specificity</th>
<th>Test meal</th>
<th>Gold standard</th>
<th>$^{13}$C urea dose (mg)</th>
<th>Cutoff level ($^{13}$C‰)</th>
<th>Fasting</th>
<th>Time sample collection (mins)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rowland et al (19)</td>
<td>100%/97.6%</td>
<td>None</td>
<td>Culture of biopsy or histology</td>
<td>50&lt;50 kg</td>
<td>75&gt;50 kg</td>
<td>3.5</td>
<td>&gt;2 h</td>
</tr>
<tr>
<td>Kalach et al (27)</td>
<td>100%/98.3%</td>
<td>Citric acid</td>
<td>Culture of biopsy</td>
<td>75</td>
<td></td>
<td>3.44</td>
<td>Overnight</td>
</tr>
<tr>
<td>Vandenplas et al (24)</td>
<td>96%/93%</td>
<td>Ice cream</td>
<td>Culture of biopsy</td>
<td>2/kg (100 mg maximum)</td>
<td>2.5</td>
<td>&gt;26 h</td>
<td>10, 20, 40, 60</td>
</tr>
<tr>
<td>Kato et al (25)</td>
<td>100%/96.3%</td>
<td>None</td>
<td>Culture of biopsy or histology</td>
<td>3/kg (100 mg maximum)</td>
<td>4.5</td>
<td>&gt;2 h</td>
<td>10, 20, 30, 40</td>
</tr>
<tr>
<td>Delvin et al (4)</td>
<td>100%/100%</td>
<td>Citric acid</td>
<td>Histology</td>
<td>50&lt;15 kg</td>
<td>75&gt;15 kg</td>
<td>3.0</td>
<td>Overnight</td>
</tr>
</tbody>
</table>

$\delta^{13}$C‰ Delta $^{13}$C/million

defection. It may also become an important diagnostic tool in the assessment of children with nonspecific, recurrent abdominal pain.

Most studies that have used the $^{13}$C UBT in children have been published since 1997. These studies have evaluated the prevalence of $H$ pylori infection in different pediatric populations (5-14) and spontaneous clearance of infection in preschoolers (7,15,16), as well as the success of eradication after treatment (4,17-19).

Few studies have validated the test for use in the pediatric age group (Table 1).

**METHODOLOGIC CONSIDERATIONS**

Investigators have compared the accuracy of histological examination for $H$ pylori with that of $^{13}$C UBT. However, the $^{13}$C UBT has not been standardized among various study centres. Difference in testing variables include presence of a fasting state, dose of urea labelled with $^{13}$C, delta cutoff level of $^{13}$C carbon dioxide, use of a variation of test meals to delay gastric emptying and the time of collection of breath samples.

**Fasting:** The duration of withholding food has varied from 2 h to overnight. Rowland et al (19) demonstrated that eating just before the $^{13}$C UBT decreases the sensitivity of the test to only 50%. They also showed that tests carried out after an overnight fast and those undertaken between 1 and 2 h after a meal are both highly sensitive and specific. However, the mean excess of $^{13}$C carbon dioxide over baseline values is lower in fed than in fasting patients. This finding implies that food in the stomach reduces the interaction between $H$ pylori urease and the urea substrate.

**Test meal:** Food has been used to slow gastric emptying to enable the $^{13}$C urea to be in sufficient contact with the urease enzyme of the organism. Tests meals employed have varied from none to citric acid to ice cream. Dominguez-Munoz et al (20) studied different test meals in adults (0.1 N citric acid solution, semiliquid fatty meal and semiliquid meal) and showed 100% specificity of the $^{13}$C UBT for all test meals, with identical sensitivities. However, the citric acid solution gave higher $^{13}$C carbon dioxide delta peak values. Use of a citric acid test meal increases the separation of excess delta $^{13}$C carbon dioxide values between $H$ pylori infected and uninfected patients, thereby making the test more robust, with fewer test results subject to misclassification (4).

This benefit must be weighed against the poor taste of citrate, which can reduce compliance, particularly in young children. Patients ingesting a fatty meal have a higher intragastric pH than those taking citric acid. The $H$ pylori urease enzyme is likely to be more active at the lower pH obtained with citric acid.

**Cutoff values:** $^{13}$C carbon dioxide is naturally present in expired air. In patients with a positive breath test result, the level of $^{13}$C carbon dioxide may increase by only one part/1000. Therefore, the most appropriate technique to measure $^{13}$C carbon dioxide is mass spectrometry, which can detect particles of a given molecular weight with extreme precision.

The amount of $^{13}$C carbon dioxide expired in the breath varies according to the quantity of $^{12}$C carbon dioxide expired. Results are generally expressed, therefore, as a ratio of $^{13}$C to $^{12}$C. The amount of $^{13}$C carbon dioxide in breath varies according to the subject’s diet. Individuals eating a $^{13}$C-rich diet (as contained in corn and other maize products) have a higher level of basal $^{13}$C carbon dioxide excretion. The measurement of $^{13}$C enrichment based on the $^{13}$C to $^{12}$C ratio is independent of the volume of expired air. In principle, it is, therefore, a much more reliable test in pediatric patients than is the $^{14}$C UBT, which depends on expired air volume in a fixed time frame (21,22).

**Results of the $^{13}$C UBT are given as $^{13}$C enrichment, which is expressed as the delta $^{13}$C/million ($\delta^{13}$C‰), or delta $^{13}$C carbon dioxide excretion/million at 30 mins compared with delta $^{13}$C carbon dioxide excretion at baseline:**

$$\delta^{13}$C‰ = \frac{^{13}$C/^{12}$C sample - ^{13}$C/^{12}$C PDB \times 10^6}{^{13}$C/^{12}$C PDB}$$

where PDB is the Pee Dee Belemnite unit.

Mion et al (23) defined a cutoff value of 3 $\delta^{13}$C‰. We used the same cutoff level (4) and found it to be both highly specific and highly sensitive (100%). Vandenplas et al (24) reported a false positive rate of 17% using a cutoff level of 2.5 $\delta^{13}$C‰, whereas Rowland et al (19) observed 100% sen-
sitivity and 97.6% specificity at 30 mins, using a cutoff level of 3.5 \( {^{13}}C \)%.

**Collection of samples:** The time at which breath samples are collected after ingestion of \( {^{13}}C \) urea varies among the published studies. Rowland et al (19) found that samples obtained at 15 mins are less specific than those performed at 30 mins. This difference probably occurs because of the interference with the test by urease-producing bacteria in the oral cavity. Similarly, if specimens are obtained at 60 mins after ingestion, there is a significant loss in specificity (19).

**ACCURACY OF \( {^{13}}C \) UBT**

The \( {^{13}}C \) UBT has not been standarized among the various pediatric studies. Table 1 compares the differences in sensitivities and specificities reported to date.

Specificity and sensitivity were high in each study. Vandenberg et al (24) obtained a higher rate of false-positive results (17%) when using a cutoff value of 2.5 \( {^{13}}C \)% and culture of the organism as the gold standard. The high number of false-positive results suggests that the cutoff value of 2.5 \( {^{13}}C \)% employed is too low.

**Test meal:** Rowland et al (19) found that a test meal is not necessary in children. Six infected children were given ice cream and a cola drink immediately before the \( {^{13}}C \) UBT. There were three false negative results at 30 mins when a false-negative one to two months after the completion of eradication therapy remained free of infection 12 to 19 months after therapy.

**Fasting:** Interestingly, Rowland et al (19) showed no differences in sensitivity and specificity between \( {^{13}}C \) UBT carried out after an overnight fast and tests undertaken between 1 and 2 h after ingestion of a test meal.

**Reinfection rate:** Using the \( {^{13}}C \) UBT, Kato et al (25) studied the rate of reinfection after eradication therapy against \( H. pylori \). Twenty-three successfully treated patients (biopsy negative one to two months after the completion of eradication therapy) remained free of infection 12 to 19 months after therapy. Thereafter, the reinfection rate was 2.4%/patient-year. In contrast, Rowland et al (26) have shown that reinfection is rare in children older than five years of age (1.35%/patient-year), whereas it occurs frequently in preschool children under five years of age (60.6%/patient-year).

**SUMMARY**

The \( {^{13}}C \) UBT is very attractive as a diagnostic test because it is highly accurate, easy to perform, noninvasive and nonradioactive. Unfortunately, it cannot be performed in many centres because mass spectrometry is not widely available. However, the stability of \( {^{13}}C \) carbon dioxide offers the advantage of being able to send breath samples over long distances. A 12-month delay in delivery of a sample to the laboratory does not modify the results of the \( {^{13}}C \) UBT (unpublished data, JL Brazier). Extremes in temperature in either the summer or winter also do not influence test results (unpublished data, JL Brazier). Ten replicates can be measured from one 10 mL breath sample collected into a vacuum-tainer tube. However, standarization of the \( {^{13}}C \) UBT among reference laboratories is necessary and should become an important priority.

In a recent pediatric study (4), 12 (15%) of 79 patients tested were infected with \( H. pylori \), as shown by both the \( {^{13}}C \) UBT and histological examination. Among these 12 children, nine were first-generation Canadians born to immigrant parents, eight of 12 received triple therapy and seven of these were retested by \( {^{13}}C \) UBT one month or more after treatment. At reassessment, four of seven were \( {^{13}}C \) UBT-negative after therapy – findings comparable with those reported by Rowland et al (19). Thus, the \( {^{13}}C \) UBT may be used for the diagnosis of \( H. pylori \) infection, as well as for the evaluation of eradication after therapy.

**CONCLUSIONS**

Further study is needed to evaluate the impact of \( H. pylori \) infection in children. The long term results of treatment remain uncertain, as do the epidemiological aspects of this infection in various pediatric populations. The \( {^{13}}C \) UBT will prove to be very helpful as an accurate and reliable diagnostic tool in these prospective studies.


