12: Practical diagnosis of Helicobacter pylori

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Introduction

The idea of antibiotic treatment for peptic ulcer disease was introduced in 1984 but execution of the concept proved difficult. Whereas therapy with H2 blockers could be instituted on the same day as the endoscopy, the final results of mucosal biopsies for *Helicobacter pylori* were available only after 2–3 days. Thus patient management could not be decided immediately, and an extra clinic visit was usually necessary. Although biopsy detection of *H. pylori* is still the "gold standard," accurate non-invasive tests promise to make diagnosis and treatment of the infection a simple event for most physicians. This chapter reviews currently available diagnostic methods, and outlines the role for each method in various types of practice. Algorithms used to diagnose *H. pylori* at the University of Virginia are shown in Figs. 12.1 and 12.2.

Where do you find *H. pylori*?

Early papers on *H. pylori* isolation stated that the bacterium was more prevalent in the antrum of the stomach than the body mucosa [1]. Subsequent reports indicate that, while larger numbers of *H. pylori* are present in the antrum, organisms are also present in body mucosa, and are easily detected in both regions of the stomach [2–5]. This issue has specifically been addressed by Bayerdorffer *et al.* [6]. In 50 patients undergoing endoscopy, 32 of which were *H. pylori*-positive (HP+), biopsies were taken from 10 locations to estimate the concordance between gastritis and *H. pylori* colonization throughout the stomach. Active gastritis (both acute and chronic inflammation) was present in 100% of 32 patients with *H. pylori*, but in none of the 18 patients without *H. pylori*. Although not every biopsy sample from HP+ patients contained *H. pylori*, multiple samples improved the sensitivity of histology to almost 100%, as shown in Fig. 12.3. It can also be seen that both the antrum and body mucosa were colonized in nearly all patients. Bayerdorffer concluded that the distribution of *H. pylori* usually exceeded the distribution of active gastritis, and that the bacterium could be found in most areas of the stomach.

Our experience is similar. In 130 consecutive HP+ patients biopsied at the University of Virginia, 128 had *H. pylori* in both the antrum and body mucosa (two specimens were usually taken from each site). As in Bayerdorffer *et al.*'s study, we found that an extremely patchy distribution of *H. pylori* occurred when intestinal metaplasia was present. As described below, this led us to use blind suction biopsy to obtain culture specimens from some patients in whom endoscopy was not otherwise indicated.

H. pylori distribution in gastric and duodenal ulcer

The distribution of *H. pylori* may vary depending on the disease state present. Since duodenal ulcers are associated with high or normal acid secretion [7], it is not surprising that in duodenal ulcer disease the body mucosa tends to be histologically normal with lower numbers of *H. pylori* present [2, 3, 5]. In gastric ulcer disease, the body mucosa is more severely affected by gastritis and acid secretion tends to be lower [8]. This trend suggests that in asymptomatic persons without acid–peptic disease or dyspepsia, acid secretion may be lower and colonization

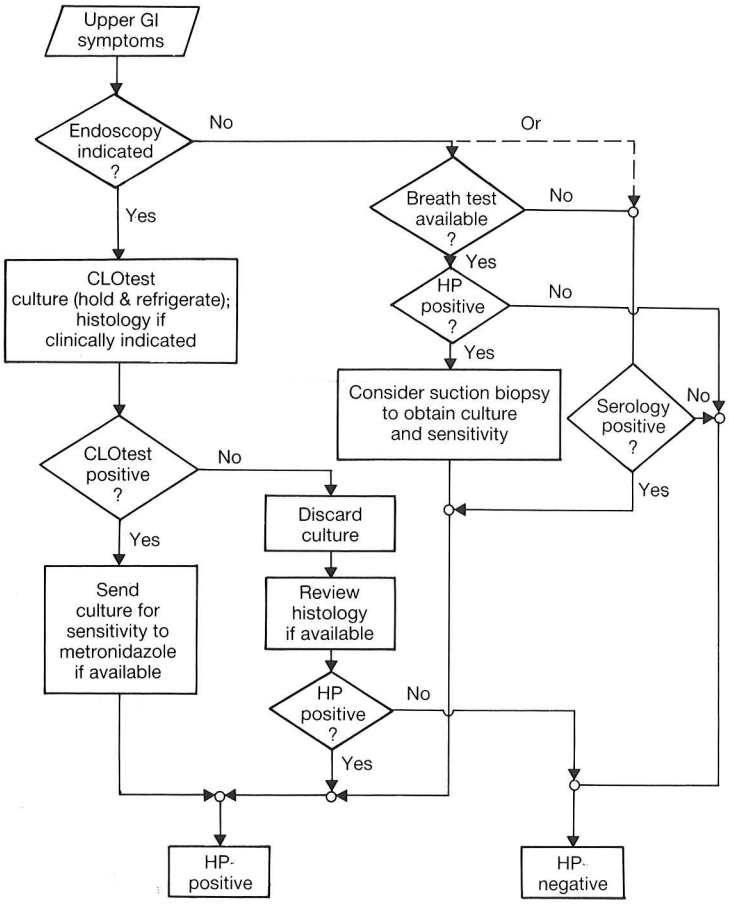


Fig. 12.1 Initial diagnosis of *H. pylori*. Note that when a CLOtest is taken, a specimen may be saved and refrigerated, so that a culture can be sent if the CLOtest is positive. This is cost effective in our hospital where the prevalence of *H. pylori* is only 40%, and most of the cultures would otherwise be negative.

with *H. pylori* may be very dense in all areas of the stomach.

The reasons for varying *H. pylori* distribution are unknown. Genetic (blood group, secretor status), environmental (diet, medications), and bacterial virulence factors (attachment, toxins) may be involved. No doubt the anatomy of the gastric mucosa is also an important factor. Antral mucosa consists of mucus-secreting epithelial cells folded into glandular structures, whereas in body mucosa the mucus-secreting cells are only on the flat surface and in the superficial portions of the gland necks. As a consequence, there is far more mucus-secreting surface present in the antrum, so a far greater bacterial load may find attachment.

H. pylori in dental plaque

Recently, *H. pylori* was found in dental plaque by Shames *et al.* [9]. The strain gave an identical restriction pattern to that seen in the stomach, suggesting that it may have seeded the gastric mucosa, or vice versa. Of concern is the consideration that, if *H. pylori* does arise in dental plaque, it is likely to be relatively infectious and be easily spread by kissing. In our 1982 study [1], I did not observe any association between poor dental hygiene and *H. pylori* infection, so at present I am skeptical that dental plaque is a major source of *H. pylori*. There is little doubt, however, that live organisms do reach the mouth in refluxed gastric juice, and could be infectious. A recent report by Majmudar *et al.* [10] described a very high isolation rate for an *H. pylori*-like organism (HPLO) from dental plaque. Urease was of course present in the dental plaques, possibly caused by the HPLO. The bacteria in question were identified as oxidase, catalase and urease positive spiral Gram-negative organisms but they await more exact identification by poly-

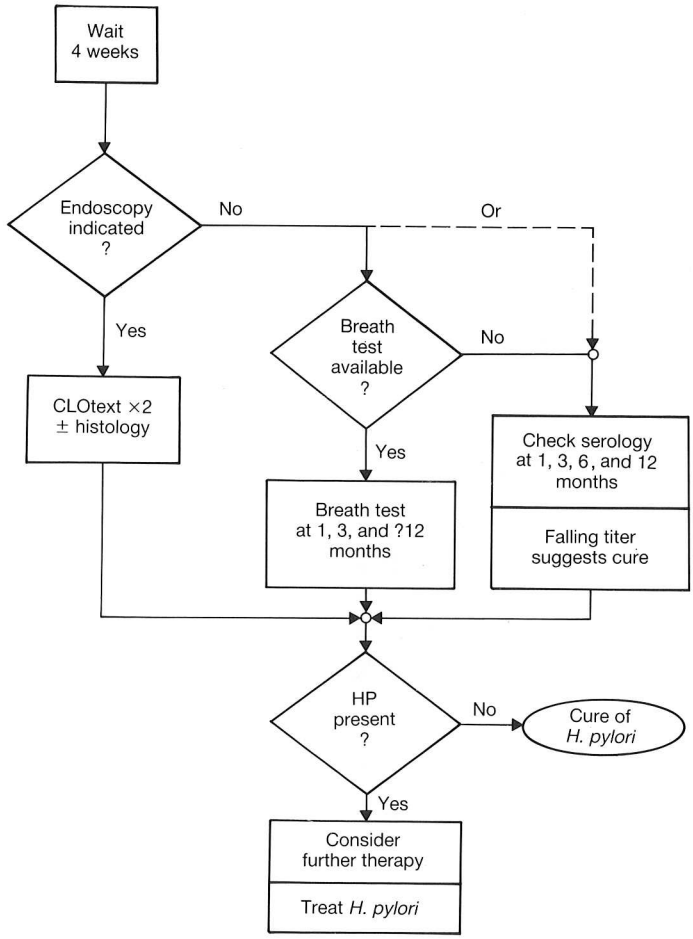


Fig. 12.2 Post-therapy diagnosis of *H. pylori*. After treatment, more care needs to be taken with detection of *H. pylori* and, if possible, serology should be replaced with a definitive test, i.e. breath test or biopsy.

acrylamide gel electrophoresis and restriction endonuclease.

H. pylori in the esophagus

The quantity of *H. pylori* in any mucosal site depends on the number of mucus-secreting epithelial cells present. Gastric mucosa, as part of Barrett's epithelium in the esophagus, may become colonized by *H. pylori*. Usually however, the amount of true gastric epithelium in Barrett's mucosa is small, the majority of the cells being of intestinal type. Usually therefore, only small numbers of *H. pylori* can be found in Barrett's epithelium, and inflammation is not associated with presence of the bacterium [11].

An exception to this may occur in Barrett's ulcers. Endoscopically, these may appear identical to gastric ulcers and are often difficult to heal. In some of these ulcers gastric mucus-secreting mucosa is the predominant type, in which case *H. pylori* and gastritis may play a pathogenic role as they do in the stomach. A child with Barrett's ulcer of the esophagus and associated *H. pylori* has been reported by De Giacomo *et al.* [12]. In this case large numbers of the bacteria were present and healing was achieved with a combination of acid-reducing therapy and amoxicillin.

H. pylori have recently been described adhering to the so-called "inlet patch," a small island of heterotopic gastric mucosa present in the esophagus at about the level of C6 in some persons [13]. Borhan-Manesh & Farnhum [14] noted that nine of 22 patients with *H. pylori* and gastric *H. pylori* infection also had infection of the upper esophageal gastric mucosa, some-

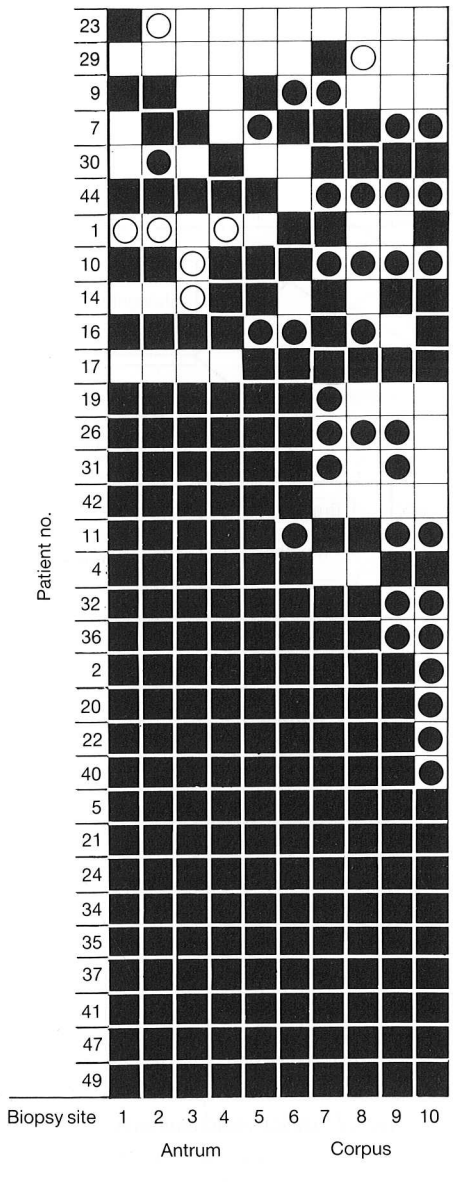


Fig. 12.3 Distribution of *H. pylori* and gastritis in the stomach of 32 patients with *H. pylori*. ■, biopsy sites with HP+ active chronic gastritis; ○, HP− active chronic gastritis; ●, HP+ sites with normal mucosa; □, neither gastritis nor *H. pylori* found. (From Bayerdorffer *et al.* [6].)

times in association with typical active gastritis. This may be a relevant factor in the design of therapy since an oral medication excreted in saliva would be needed to reach such an *H. pylori* colony and eradicate the organisms. Conversely, swallowed bismuth tablets might be less effective at *H. pylori* eradication in such patients.

H. pylori in the duodenum with metaplasia and heterotopia

H. pylori is often found in the duodenum, but almost always in association with gastric epithelium. Gastric tissue takes two forms in the duodenum. The most common form is an antral type of epithelium referred to as gastric metaplasia (as seen in Fig. 12.4). Gastric metaplasia is present in 92% of duodenal ulcer borders and is seen elsewhere in the duodenal bulb 50% of the time. When *H. pylori* is present in the antrum, associated gastric metaplasia in the duodenum will be colonized 50% of the time.

Since duodenal gastric metaplasia is patchy and cannot be identified through the endoscope, biopsy of the duodenal bulb has a very high sampling error, and may miss both gastric metaplasia and *H. pylori*. For this reason diagnosis of *H. pylori* cannot be excluded with a duodenal biopsy. In any case, whenever patchy *H. pylori* is present in the duodenum, bacteria are always present in the antrum and are more easily diagnosed by gastric biopsy.

At the University of Virginia, we have also found gastric metaplasia in 60% of patients with non-ulcer dyspepsia. In the study reported by Frierson *et al.* [15], two biopsies were taken from the proximal duodenal bulb in patients with non-ulcer dyspepsia. In addition, patients with a history of peptic ulcer disease were excluded from the study. Frierson found that gastric metaplasia was only associated with duodenitis when *H. pylori* was present, in the antrum if not in the duodenum. Gastric metaplasia was so prevalent that it was almost a normal finding, and was only associated with duodenitis when *H. pylori* infection coexisted. These findings support those of Wyatt *et al.* [16], who showed a near 1 : 1 relationship between duodenitis and *H. pylori*–gastric metaplasia. Frierson and others have also noted that *H. pylori* is never seen attached to duodenal epithelium unless gastric metaplasia is present.

A second form of gastric mucosa, gastric heterotopia, may also be present in the duodenum. Gastric heterotopia is true body-type

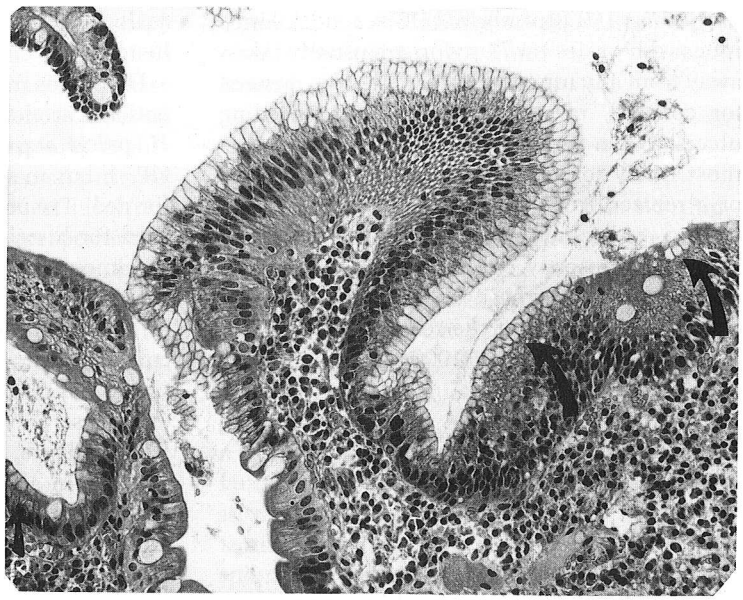


Fig. 12.4 Gastric metaplasia in the duodenal bulb. Most of the large villus is lined by gastric metaplasia, as readily seen in this H&E-stained section. Much smaller foci of gastric metaplasia are also present (arrows). H&E, × 250. (From Frierson *et al.* [15].)

acid-secreting mucosa, rather than mere mucus-secreting antral-type mucosa seen in gastric metaplasia. The role of gastric heterotopia in duodenal ulcer is unknown, but potentially important. According to Carrick *et al.* [17], islands of gastric heterotopia are found in 30% of biopsy samples taken adjacent to duodenal ulcers. If the bulb was sprayed with congo red and the patient was given pentagastrin prior to endoscopy, Carrick *et al.* could demonstrate functional acid-secreting tissue in over two-thirds of duodenal ulcer patients. So far the association between gastric heterotopia and duodenal ulcer has not been confirmed in other centers.

H. pylori in Meckel's diverticula and in the rectum

The remarkable affinity which *H. pylori* has for gastric epithelium is further demonstrated when gastric metaplasia or gastric heterotopia occurs in the gut distal to the duodenum. De Cothi *et al.* found gastric metaplasia in 13 Meckel's diverticula [18]; eight showed gastritis, and *H. pylori* were seen in half of these. Morris *et al.* have also reported *H. pylori* in Meckel's diverticula [19]. An even rarer finding of *H. pylori* in the rectum was reported by Dye *et al.* [20]. They described a woman with gastric heterotopia in a small reduplication cyst of the rectum. A *Campylobacter*-like organisms test (CLOtest) of the tissue was strongly positive, and histology revealed typical active chronic gastritis with CLO present on gastric body-type mucosa. Electron micrographs were compatible with *H. pylori* colonization.

Thus, the histology of *H. pylori* infection is the same, regardless of its location. In addition, *H. pylori*-associated disease (gastritis) may be expected in any location where gastric mucosa is found, i.e. throughout the gut. Finally, the stomach is the major reservoir of *H. pylori* only because gastric epithelial cells are concentrated there. The presence of *H. pylori* in the rectum is strong evidence that live *H. pylori* organisms are present in the feces, that *H. pylori* can easily compete with colonic flora for the juxtamucosal site, and that acid is not necessary for *H. pylori* to colonize gastric-type mucosa.

When to biopsy

Before taking a gastric mucosal biopsy, the risks and benefits need to be assessed. The only contraindication to a small pinch biopsy is a severe bleeding disorder. Usually patients can be safely biopsied if the prothrombin time is not

more than 150% of normal (15 seconds). Since mucosal biopsies for *H. pylori* are usually taken away from any mucosal lesions, there is no need for concern, even in patients with bleeding ulcers. When significant bleeding occurs, it is most likely to be from a biopsied ulcer border or a rebleed from an existing lesion. It is my practice to include mention of a gastric biopsy in every endoscopy consent form, and to state the risk of hemorrhage or perforation from a biopsy as 1000 : 1, but the real risk is certainly much smaller, perhaps 50 000 : 1.

Obtaining a culture without doing endoscopy

Since *H. pylori* organisms are distributed throughout the gastric mucosa, blind biopsy of the gastric mucosa may be used to obtain a culture of *H. pylori* when endoscopy is not otherwise necessary. Contraindications to blind biopsy are the presence of a coagulopathy or presence of esophageal diverticula pouches, etc. For this procedure, the patient fasts overnight and attends the gastroenterology clinic at 9 am. The patient sits on the endoscopy table and the throat is sprayed with local anesthetic. After measuring the length of the patient from teeth to xiphoid, a "Quinton" suction biopsy tube and capsule (diameter 3.5 mm) is passed through the patient's mouth, and the location within the stomach is checked by injection of air and auscultation over the stomach. Usually a little resistance is felt at the esophagogastric junction, which is helped by a drink of water. In most cases some gagging and eye-watering occurs, but patients usually tolerate the tube for 10 minutes without excessive difficulty.

A negative pressure is then drawn on the tube, and after the tube becomes obstructed with gastric mucosa, a cable pulls a small guillotine within the biopsy capsule and (usually) a mucosal biopsy is obtained. The process may be repeated so that two or even three samples can be taken with one pass of the tube. The mucosa should be examined with a magnifying glass after removal from the device. Usually gastric mucosa is orange because of blood within it, and small dots (gastric pits) can be seen on its surface. Even though the hole in the biopsy capsule is only 2 mm wide, suction biopsies tend to be quite large and can be divided with a scalpel to provide specimens for culture and histology.

Using this method, we studied 13 consecutive patients attending the gastritis clinic in whom *H. pylori* status was known (nine HP+, four HP−), but in whom culture had not been performed. Tissue was sent primarily for culture, then for histology and CLOtest, depending on the amount obtained. The tube was successfully passed without sedation in all 13 patients, but in three patients no biopsy material was retrieved. Of the 10 patients successfully biopsied, tissue was obtained for culture and histology in every case, and for CLOtest in seven. In the nine HP+ patients, histology revealed *H. pylori* and gastritis in all nine (100%), and *H. pylori* was cultured in eight (89%). CLOtest was positive in all six HP+ patients tested (100%), but in the other three HP+ subjects insufficient tissue was obtained to do a CLOtest. According to the histology, biopsy samples were obtained from the antrum in three patients (33%) and from body mucosa in six (66%). In two patients who underwent endoscopy after the suction biopsy, suction marks were seen on the greater curve in the mid-body mucosa.

One week after the test, patients completed a mailed questionnaire to evaluate their impression of the procedure. Patient scores, representing grades from "no difficulty" to "great difficulty", ranged between 0 and 10, with an average score of 2.6. Of 10 patients who had undergone upper endoscopy in the past, seven preferred the suction biopsy. In most patients, therefore, suction biopsy can obtain sufficient material to perform culture and histology for *H. pylori*. In this small series the sensitivity of the test was 100% in persons from whom tissue was obtained. We suggest that this technique may be a useful way for non-endoscopists to easily obtain tissue and culture for *H. pylori* studies. Since the test appears quite sensitive (probably 90% for a single gastric mucosal biopsy), it may also be used to screen for the infection or follow up patients after treatment.

Staining of fresh tissue

Gram staining of a smeared gastric biopsy was the first rapid method used to diagnose *H. pylori* [21]. With a sensitivity of 80–90%, Gram stain-

ing added at least 60 minutes to the endoscopy session while the sections were stained and examined. Pinkard *et al.* [22] found that phase contrast microscopy of a squashed mucosal biopsy was a faster (5 minutes) and highly sensitive method (100% in 44 infected patients) of detecting the spiral organism. In ideal circumstances, the above tests were performed immediately, in the endoscopy room, by a trained technician. Whereas this was possible in a research setting, it was not practical for general use. Stains of fresh tissue have been superseded by the biopsy urease test.

Biopsy urease test

Langenberg *et al.* were the first to note the vigorous urease production of *H. pylori* [23], and Owen *et al.* reported that they could rapidly identify cultures of the new spiral organism by an immediate color change in urease detection agar (Christensen's medium) [24]. For weaker urease positive organisms (e.g. *Proteus*), the bacterium had to be cultured in the agar and a color change only occurred after many hours.

The idea of placing a gastric biopsy into such a medium was patented in 1985, and an enhanced urease test became available as the CLOtest in 1988 [25]. The rapid urease test is highly sensitive (90–95%) and very specific, as evidenced by the large number of publications on the subject (18 in 1989 alone) [26–34]. In most studies, a single biopsy sample for the urease test has been compared with a "gold standard" of multiple biopsies for histology and/or culture. The question of which is most sensitive, a single urease test or a single sample for histology, was addressed by Dye *et al.* [35]. They found that the rapid urease test (CLOtest) was more sensitive than histology or microbiology when these other modalities were only allowed a single biopsy sample. In addition, whereas histology and culture were expensive, labor intensive, and gave a result in 24–72 hours, the CLOtest could be read in the endoscopy room by the nurse, with 90% of positive results available before outpatients left the hospital.

Urease tests have been reviewed in Chapter 7. In brief, a faster urease test can be produced if occasional false-positive results are accepted. The CLOtest is buffered so that false-positive results are rare, but this limits its sensitivity to around 95% per biopsy.

Histological stains

Initial *H. pylori* studies used the Warthin–Starry silver stain, but many laboratories could not obtain consistent results with this difficult method. Instead, most changed to simpler stains such as the Giemsa [36], Gimenez [37] or acridine orange [38, 39]. All these are simple one-step stains which can be produced for minimal cost ($20 US) at the same time as routine hematoxylin and eosin (H&E) stained sections. They are almost as sensitive as a good silver stain.

A good pathologist will suspect *H. pylori* whenever the H&E section shows gastritis, and the bacteria are often easily visible in such sections at ×500 magnification. If the bacteria can be identified, further special stains are unnecessary. To save expense, an extra paraffin section can be cut during initial processing of the tissue, and only stained if there is doubt as to the presence or absence of *H. pylori*.

Culture of *H. pylori*

Transport of specimens

There is no need to culture the gastric biopsy immediately in the endoscopy room. At the University of Virginia in 1987 we compared isolation rates from immediate plating and gassing of biopsies with standard laboratory processing by a laboratory technician. We found that heavy contamination often occurred when the specimen was plated in the endoscopy room. Isolation rate was slightly better if the specimen was merely placed in a drop of saline, refrigerated and sent to the laboratory up to 2 hours later.

Achlorhydria

Endoscopists should be aware of the special problems with *H. pylori* culture. The normal stomach is sterile but patients with achlorhydria or gastric stasis, or who take omeprazole, may have heavy colonization of the stomach

with aerobic and anaerobic flora. Because *H. pylori* grows slowly, it may be overrun by more vigorous bacteria and thus not be cultured. Therefore, when one of the above complications is suspected, selective culture plates should be used with antibiotics such as vancomycin, nalidixic acid, cefsulodin, and amphotericin present in the medium. A Skirrow's plate used for *Campylobacter* isolation is a reasonable alternative in most laboratories. In a research setting, sensitivity of 95–99% can be obtained using selective media for the isolation of *H. pylori* [29].

Several investigators have noted improved growth of *H. pylori* on fresh media rather than media from commercial sources [40], and most have emphasized the necessity for the atmosphere to be very humid. Finer points of culturing *H. pylori* are discussed in Chapter 7.

Radiographic findings in gastritis

According to de Lange *et al.* [41, 42], the radiographic features of gastritis vary, and the diagnosis is sometimes difficult to make because the findings may be subtle. However, to improve the detectability of the abnormalities, it is important that a state-of-the-art technique be used for the radiographic examination of the upper gastrointestinal tract. The biphasic contrast technique is considered the most sensitive method to detect abnormalities in the stomach, including gastritis. This radiographic examination consists of two techniques, a double-contrast and a single-contrast method combined in one study. It is important to use this technique for the evaluation of gastritis, because some features are only visible on double-contrast radiographs, whereas other findings are best seen on single-contrast films during compression of the stomach (Figs. 12.5, 12.6). For optimal results it is essential to use a medium-dense barium suspension, and glucagon (0.2 ml) is administered intravenously for gastric distension.

Radiographically, gastritis may be present when the following findings are demonstrated: erosions, nodules, polyps, transverse folds in the antrum, crenulation, antral spasm, S-shaped antrum, paucity of folds, and coarse areae gastricae (Figs. 12.5, 12.6). Not all of these findings may be present at the same time, and usually only one or two features are demonstrated. Erosions and coarse areae gastricae are

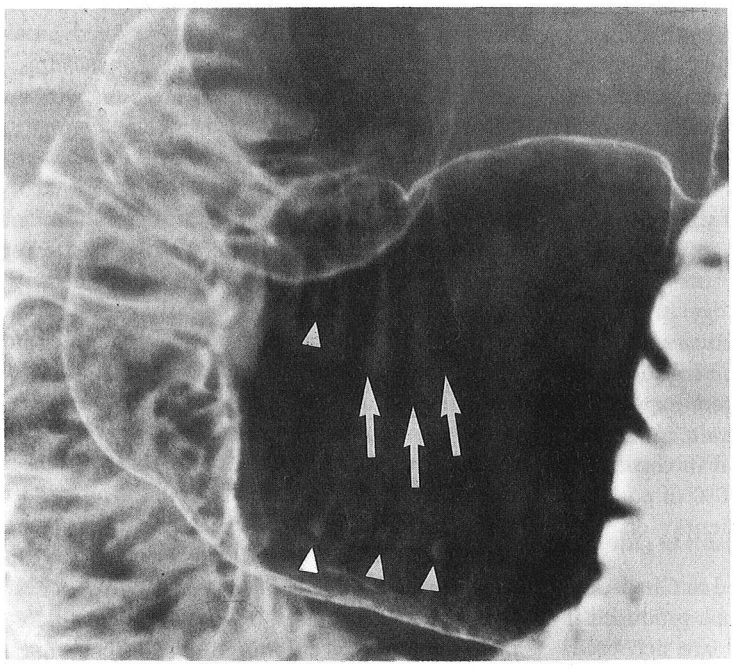


Fig. 12.5 There are multiple transverse antral folds perpendicular to the lesser curvature (⇧). Erosions are also present (arrowheads); some of the erosions are aligned along the folds. (From de Lange [41].)

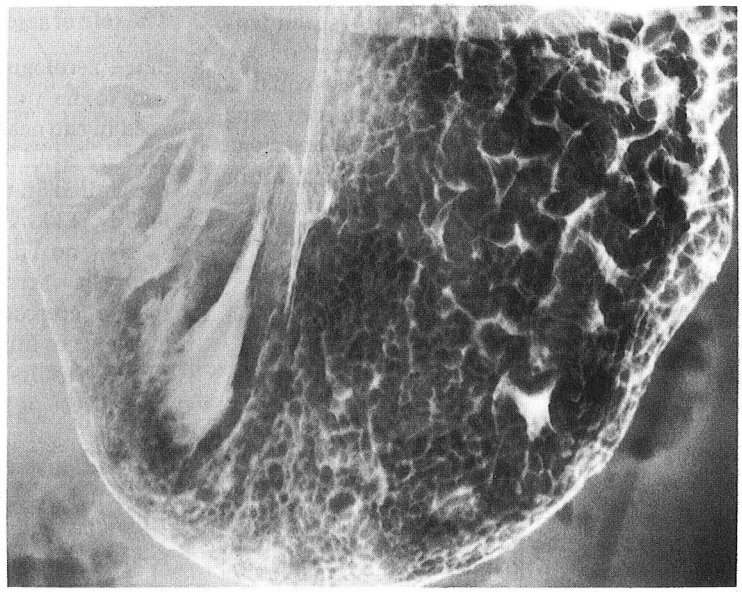


Fig. 12.6 Coarse areae gastricae. The individual mucosal units have an irregular shape and are pronounced. They measure 5–6 mm in diameter. Note the presence of transverse folds and thickened antral folds. (From de Lange [41].)

usually best seen during the double-contrast phase of the biphasic examination, whereas nodules and polyps are usually demonstrated during the single-contrast graded-compression phase. Transverse folds, S-shaped antrum, antral spasm, and crenulation are demonstrated on both single- and double-contrast films.

It is impossible to distinguish radiographically between acute and chronic gastritis, as both entities may present with similar findings. However, findings such as transverse folds, antral deformity, nodularity, and polyps are in favor of chronic gastritis. Erosions can be seen in both acute and chronic gastritis, but demonstration of transverse folds together with erosions (Fig. 12.5) is in favor of chronic disease.

When performed properly by a skillful and experienced radiologist, the biphasic contrast examination may be a sensitive, non-invasive test for the evaluation of the stomach for gastritis. As the findings may be subtle, careful and meticulous evaluation of the radiographs is necessary to detect the various features.

Immunologic detection of *H. pylori* in biopsy material

A rapid ELISA method was described by Engstrand *et al.* [43] for detecting *H. pylori* rapidly in fresh biopsy material. Biopsy specimens were smeared onto a glass slide which was incubated with an IgG mouse monoclonal antibody directed towards a 23 kDa *H. pylori* antigen. After washing, the presence of adherent mouse IgG was detected by staining with an anti-mouse antibody conjugated to fluorescein. The method was at least as sensitive as silver stain of multiple biopsy specimens, and could be performed in 1 hour.

Shadowen & Sciortino have used latex agglutination to detect *H. pylori* antigen in fresh biopsy specimens [44]. Briefly, latex beads are coated with monoclonal antibody to *H. pylori*, a biopsy or mucus sample is mixed with the latex suspension, and agglutination occurs if *H. pylori* antigens are present in the sample. The sensitivity of the method equals that of histology (95–100%).

A third method, which Cartun *et al.* [45] have found to be highly sensitive and specific for *H. pylori*, is to stain histologic sections with monoclonal antibody to *H. pylori*. The monoclonal antibody used is commercially available and the methodology is standard in most histology laboratories.

The advantage of immunologic methods is that they provide positive identification of *H. pylori* organisms without culture, and are unaffected by similar-looking commensal

bacteria visible in some histology specimens. The sensitivity of immunologic methods on a single biopsy specimen is at least equal to careful examination of a silver-stained specimen, and in some hands exceeds histologic methods [45]. At present, however, immunologic detection of *H. pylori* in biopsy material is a research tool rather than a routine method of diagnosis.

Serologic tests for *H. pylori*

After 1984, many microbiologists developed serologic tests for *H. pylori*. The first of these, reported by Jones *et al.* [46], used a complement fixation test similar to those described for the detection of antibodies to *Campylobacter jejuni*. Antibodies to *H. pylori* were usually present in high titer and the sensitivity of the early tests easily reached 85%. In Western Australia we produced a passive hemagglutination test using a supernatant of sonicated *H. pylori* organisms to coat type O human red blood cells. After washing in phosphate-buffered saline and resuspending the cells in dilute calf serum, the cells survived for 5 days. The test only took 20 minutes to complete and gave a sensitivity of 90–95% and a specificity of 83% [47, 48].

After 1984, investigators detected *H. pylori* antibodies with enzyme-linked immunosorbent assays (ELISAs), using commercially available reagents and standard methodology [49, 50, 51]. The sensitivity of the ELISA can be increased by selecting the most antigenic *H. pylori* proteins to coat the wells. In the case of *H. pylori* these are flagella, surface-exposed urease, and attachment proteins such as the *N*-acetylneuraminyllactose-binding hemagglutinin (a lectin) described by Evans *et al.* [52]. ELISA tests can be made very sensitive (few false-negatives) or specific (few false-positives) by adjusting the upper limit of normal for HP– patients. Many commercial kits are in development and some of the technology is protected by patents in the United States. As yet these serologic tests have not been evaluated by independent investigators, so their sensitivity and specificity in various patient groups are poorly defined.

The role of serologic tests in management

Since serologic tests offer a cheap and rapid way to diagnose *H. pylori*, they will be widely used in the near future. Already *H. pylori* ELISA kits are available from commercial sources in the United States, United Kingdom, and Europe. Ideally, ELISA tests should be very sensitive, so that no HP+ patients are missed during a serologic screen. Strongly positive results in the correct clinical setting are sufficient evidence to initiate therapy, whereas borderline ELISA results should be confirmed with a breath test or biopsy before treatment (see Fig. 12.1).

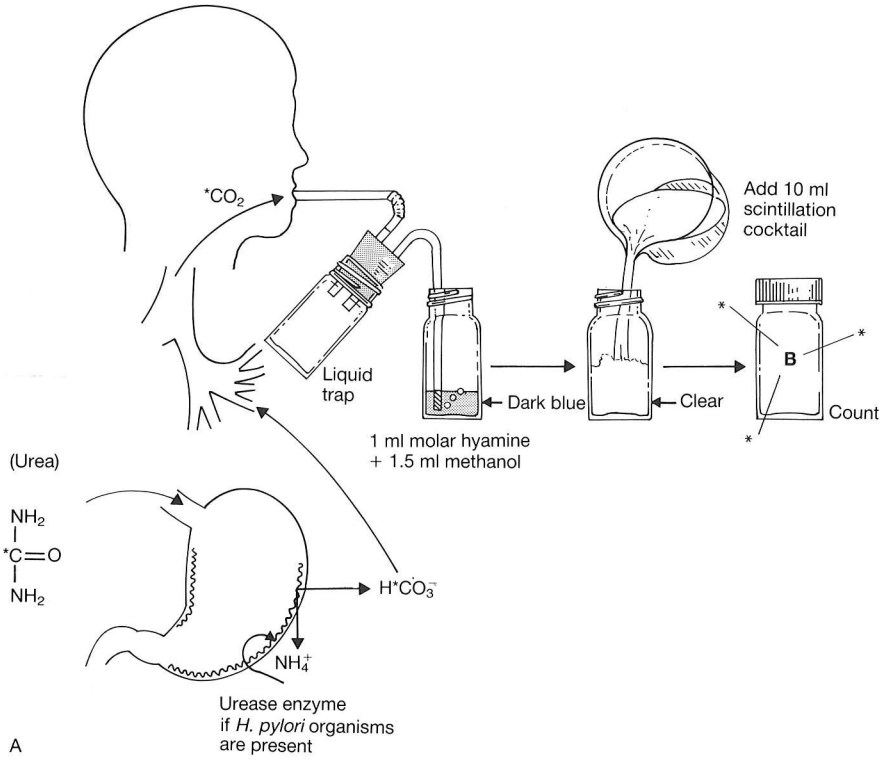
Some physicians believe that a diagnosis of duodenal ulcer is reason enough to commence *H. pylori* therapy, without further diagnostic tests. I am skeptical of this practice, because in many patients the diagnosis of "duodenal ulcer" has never been proved or was made in the distant past. Often, so-called ulcer symptoms are due to esophageal reflux disease or residual dyspepsia, persisting long after *H. pylori* has disappeared. Suggested algorithms used to sort out such patients are shown in Fig. 12.1 and Chapter 13.

Serologic follow-up of *H. pylori* infections has been reported by a number of investigators [53–55]. Due to large individual variations in titer, however, absolute antibody levels are a poor indicator of cure. It is our experience that, in patients who fail therapy, antibody titer falls during treatment but rebounds 4 weeks later. This makes serologic follow-up impractical in the weeks immediately following treatment.

When immediate proof of eradication is not essential, a consistently falling antibody titer over 3–6 months is a useful indicator of bacteriologic cure. For best results, acute and convalescent sera should be tested in the same ELISA plate. As shown in Fig. 12.2, I recommend that convalescent sera be tested at 1, 3, 6, and 12 months after treatment to confirm long-term eradication of *H. pylori*, or recrudescence.

The urea breath test

The urea breath test is the most specific noninvasive way to detect *H. pylori*. Urease is not



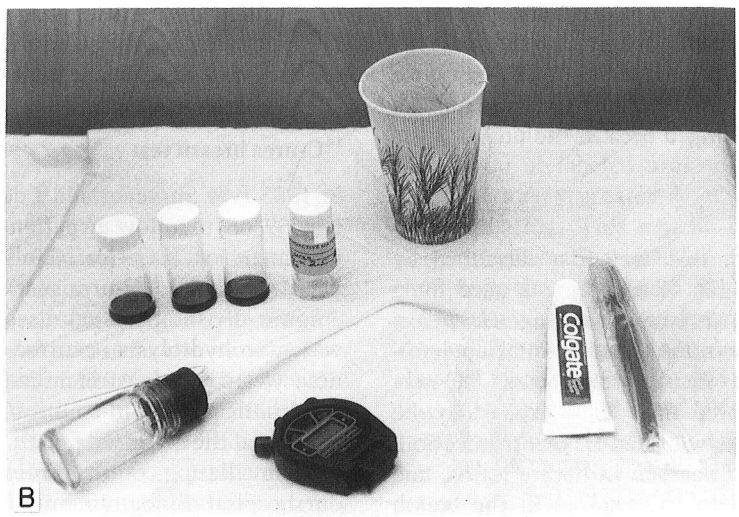


Fig. 12.7 A, if urease (*H. pylori*) is present in the gastric mucosa, isotope-labeled urea is hydrolyzed forming $^{14}CO_2$ which is expired in the breath. Patients blow through a disposable drinking straw attached to a liquid trap, into a 20 ml vial containing methanol, hyamine, and a pH indicator. The blue (alkaline) solution changes to colorless upon CO_2 saturation. Scintillation fluid (10 ml) is added to the sample, which is then counted for 5 minutes in a scintillation counter. Note: caution is required in handling hyamine solution, a strong base. (From Marshall *et al.* [65].) B, actual equipment needed for the UVA breath test (counterclockwise from right: toothbrush and toothpaste, cup, isotope, breath collection bottles containing hyamine/methanol and blue indicator, trap with drinking straw attached, stop watch).

present in mammalian cells, so the presence of urease enzyme in the stomach is proof that bacteria are present [56]. Swallowed urease from mouth bacteria is denatured below pH 4.0 [57], so it is quickly destroyed by gastric acid and cannot interfere with the test. When urease is produced beneath the gastric mucus layer, however, it is protected from luminal acid and remains fully active. The action of urease on urea in the extracellular fluid yields HCO_3^- and NH_4^+, both of which rapidly enter the blood on the mucosal side, and serve to further maintain an alkaline mucus layer on the luminal side of the mucus gel [58]. The principle of the breath test is shown in Fig. 12.7.

In order to detect gastric urease, urea labeled with a carbon isotope is swallowed by the patient. If urease (*H. pylori*) is present, urea is split into HCO_3^- and NH_4^+ at the interface between the gastric epithelium and lumen. Ammonium ion is sequestered in the gastric acid, but the CO_2 (from HCO_3^-) enters the bloodstream, where it is carried to the lungs and rapidly expired. About 50% of the urea is either absorbed through the mucosa unchanged, or leaves the stomach through the pylorus without being hydrolyzed [59, 60]. This intact urea is then excreted in the urine over the next 48 hours. In persons without *H. pylori*, very little of the isotope appears in the breath and most is excreted as unchanged urea in the urine.

^{13}C-urea breath test

The urea breath test was first described by Graham *et al.* [61]. Recognizing a need for a non-invasive means of detecting *H. pylori* in asymptomatic normal persons (for epidemiologic studies), they administered ^{13}C-urea orally and detected the $^{13}CO_2$ expired in the breath of patients who had *H. pylori* infection. About 1.1% of all carbon in nature is ^{13}C, and the ratio of ^{13}C to ^{12}C expired in the breath varies with diet and the level of physical activity [62]. This natural abundance of ^{13}C means that a relatively large dose of substrate is necessary to measurably increase $^{13}CO_2$ excretion. In practice, 2–5 mg kg^{-1} of urea (75–250 mg per patient) is used to obtain the necessary >0.6% increase in ^{13}C seen in infected patients [61, 63].

Aware of the above limitations, Graham *et al.* [61, 63] collected a baseline sample to control for intersubject expired-^{13}C variations, and then used a high-calorie liquid test meal (Sustacal) to hold the isotopic urea in the stomach. *H. pylori* urease enzyme has a K_m of around 1 mmol of urea [64], a concentration exceeded by the ^{13}C-urea test, so it was necessary to hold the reagents in the stomach for a time while the reaction proceeded. In addition, because the urea was diluted in 150 ml of liquid, mixing was required to bring the unchanged urea into contact with the gastric mucosa. Alveolar breath samples were collected in a balloon and transferred to glass test tubes for analysis in an automated isotope ratio mass spectrometer. Samples at 40–60 minutes were sensitive and specific indicators of *H. pylori* infection.

The meal-based ^{13}C-urea test has the ability to quantify accurately gastric mucosal urease, since the enzyme is saturated with substrate, and the reaction proceeds at a rate dependent on urease concentration rather than substrate concentration. By correcting for endogenous CO_2 production, the results can be expressed as the urea hydrolysis rate in μmol minute^{-1}. The disadvantage of the test is the relatively large investment in patient and technician time, the cost of the isotope, the number of samples required and, as a result, the expense.

^{14}C-urea breath test

In 1985 Ivor Surveyor and I devised a ^{14}C-urea test [59, 65] to follow up patients after antibiotic treatment of *H. pylori*. Unlike the ^{13}C-urea breath test, the ^{14}C-urea test used only a tiny amount of isotope (1 μg) dissolved in 20 ml of water, so hydrolysis occurred swiftly once the urea wetted the gastric mucosa. This made a meal unnecessary. In addition, the isotope was cheap and the collected breath could be counted in a scintillation counter which was available in our hospital. Since our initial description, the test has been further refined at the University of Virginia [66].

In order to observe the speed of urea hydrolysis and CO_2 excretion in the breath, we studied four patients with proved *H. pylori*, and two patients without *H. pylori*. After an overnight fast, a soft plastic tube was passed into the upper esophagus and 20 ml of water containing

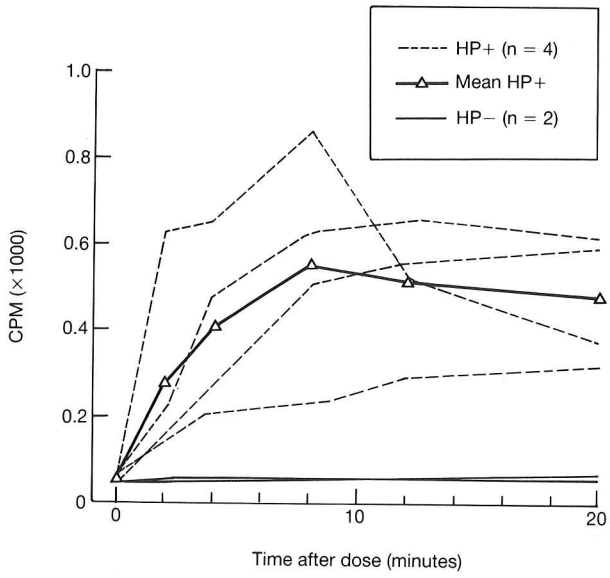


Fig. 12.8 When ^{14}C-urea is given directly into the esophagus, a gradual rise in $^{14}CO_2$ excretion is seen in HP+ subjects, easily detectable by 4 minutes (upper curve). In HP− subjects, only a very small rise in $^{14}CO_2$ excretion is seen (flat lines near bottom of graph). Dose = 37 kBq. (From Marshall *et al.* [66].)

37 kBq (1 μCi) of ^{14}C-urea was injected. HP+ patients gave diagnostic levels of $^{14}CO_2$ in breath within 4 minutes, whereas HP− patients showed no significant increase (Fig. 12.8). These results served to demonstrate that urease from bacteria in the esophagus was too weak to cause a positive breath test. In contrast, gastric urease (beneath the mucus layer) caused immediate release of $^{14}CO_2$ into the bloodstream.

Administration of labeled urea: with meals or not?

This is a controversial point among breath test experts. If urea solution is given in 20 ml water (as in our rapid test), some urea is hydrolyzed as it touches bacterial urease in the mouth. This effect of oral urease makes early readings (before 15 minutes) ambiguous. In contrast, if labeled urea is given with a high-calorie liquid meal, the isotope is very dilute as it passes through the mouth and oral urea hydrolysis is greatly reduced. The disadvantage of this method is that the breath test is usually much prolonged if a meal is given, and isotope is excreted very slowly in the breath, making it necessary to count samples for a very long time, or use a larger isotope dose [67].

When very quick results are preferred, oral contamination needs to be minimized, so that early breath samples (5–15 minutes) can discriminate between HP+ and HP− patients. To obtain a very early rise, the isotope should be given in enough water to ensure that it can be easily swallowed and reaches the stomach. A small volume of water (<20 ml) wets the gastric mucosa and pools in the antrum. Larger volumes (>20 ml) empty through the pylorus and waste the ^{14}C-urea. The disadvantage of using a small volume is that it exposes a higher proportion of labeled urea to the oropharynx, and so causes greater levels of oral urease contamination. This can be circumvented by collecting breath samples in a plastic bag and pumping them through hyamine solution, rather than having the patient blow bubbles in the solution. In the latter case, breath passes through the patient's mouth very slowly and is heavily contaminated with the products of oral urea hydrolysis. With the bag, patients blow out quickly and contamination from the oral mucosa (CO_2 being slowly released) is minimized.

Table 12.1 details the various methods used for both ^{13}C-urea and ^{14}C-urea breath tests. All are highly sensitive (85–95%) and specific (90–98%) depending on where the investigator decides to place his upper limit of normal value for HP− patients. Fig. 12.9 gives a graphical comparison of each. All ^{14}C-urea breath tests

Table 12.1 Breath tests published in the reviewed literature

Author	Isotope dose	Type of meal	Isotope given in	Collection time (minutes)
Graham [61]	^{13}C 5 mg kg^{-1}	Sustacal liquid 120 ml	Water	60
Marshall [59]	^{14}C 370 kBq	None	Water 20 ml	20
Bell [77]	^{14}C 400 kBq	Liquid 350 ml	Water 20 ml	60–120
Ormand [67]	^{14}C 370 kBq	Sustacal liquid 120 ml	Water 25 ml	20–60
Veldhuyzen van Zanten [68]	^{14}C 185 kBq	Sustacal liquid 120 ml	Water + 50 mg cold urea	60–120
Rauws [78]	^{14}C 111 kBq	Sustacal liquid 120 ml	Water	40
Eggers [60]	^{13}C 75 mg	0.1 M Citrate 200 ml	Citrate 5 ml	30–60

37 kBq = 1 μCi.

have been corrected to show counts per minute (CPM) mmol^{-1} 10 μCi^{-1} of expired CO_2. It can be seen that the highest excretion occurs when the isotope is dissolved in 20 ml of water and given without a meal [59, 65]. Similar values may be obtained by placing the urea within a quick-dissolving capsule of lactose, a method which also prevents oral urease contamination. Note that all methods which use a meal have a longer collection time although, according to Ormand *et al.* [67], a 20-minute collection will still diagnose most patients, even in meal-based tests.

Using a modified ^{13}C-urea breath test, Eggers *et al.* [60] claimed that a 20-minute collection could be used with a meal-based test. Eggers *et al.* gave 75 mg of ^{13}C-urea in 200 ml of 0.1 M citric acid solution. They also measured $^{13}CO_2$ breath collections after ingestion of labeled $NaH^{13}CO_3$ and compared the results with the ^{13}C-urea breath test. They revealed that with this method, most of the ^{13}C-urea was emptied from the stomach before hydrolysis could occur, and only 21% of the isotope was recovered as CO_2 in breath during 30 minutes' collection. Although this methodology makes comparisons difficult, it appears that retention of the isotope in the stomach is only partly achieved with the liquid meals used in the ^{13}C-urea breath test.

With ^{14}C-urea, a tiny amount of substrate and a fast reaction time (4–10 minutes) mean that prolonged retention in the stomach is not essential for diagnosis. On the other hand, if ^{14}C-urea is being used to accurately quantitate gastric urease, maximum reproducibility can be obtained if the isotope is given within a liquid meal. Veldhuyzen van Zanten *et al.* [68] have even added 50 mg of unlabeled urea to their

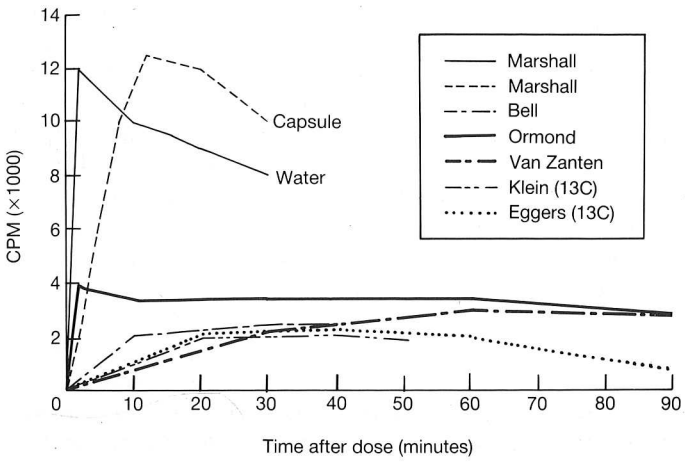


Fig. 12.9 Comparison of various breath test methods showing CO_2 excretion curves. All ^{14}C-urea tests have been adjusted to reflect a 10 μCi dose. (Further information is provided in Table 12.1.) References: [59], [66], [77], [67], [68], [63], [60].

meal-based ^{14}C-urea test to slow the reaction even further and ensure "zero order kinetics." This means that, as in the ^{13}C-urea test, the enzyme is saturated with urea and the reaction proceeds at a constant rate, proportional to the concentration of urease present.

University of Virginia method for a rapid ^{14}C-urea breath test

Lyophilized ^{14}C-urea is obtained in a 9250 kBq (250 μCi) ampule, and is reconstituted with 25 ml of sterile water; 185 kBq (0.5 ml) of the solution is pipetted into a 20 ml vial with an additional 2.0 ml of sterile water. A 0.01 ml sample of this solution is used as a batch standard. Each 2.5 ml dose is frozen at −20 °C until use. Immediately before use, the solution is thawed to room temperature and 17.5 ml of tap water is added.

Breath samples are collected in the following manner (Fig. 12.7). Patients blow through a disposable drinking straw attached to a liquid trap, into a 20 ml glass scintillation vial containing 2.5 ml of methanol, in which 1 mmol of hyamine (methylbenzethonium hydroxide) is dissolved, and to which thymolphthalein pH indicator has been added. The blue (alkaline) solution changes to colorless upon CO_2 saturation, at which time a constant amount (≈1.0 mmol) of CO_2 has been collected.

Ten milliliters of liquid scintillant (Ready-Safe; Beckman catalog #158735) is added to each vial and each sample is counted in a liquid scintillation counter (Rackbeta; Pharmacia LKB), using a quenching correction with the output expressed as CPM. A standard provided by the manufacturer indicates the counting efficiency of our machine is 93.6%. Disintegrations per minute (DPM) may thus be derived by multiplying CPM by 1.068 (100 ÷ 93.6).

A sample of 153 patients, (77 females, 76 males) took the breath test during the week of their endoscopy. After they had fasted for at least 6 hours, a baseline breath sample was collected to familiarize the patient with the technique. Each patient then removed false teeth (if present), cleansed his or her mouth with toothpaste, and was then given 185 kBq (5 μCi) of ^{14}C-labeled urea dissolved in 20 ml of water. The radioisotope was taken in a single swallow in an attempt to prevent the solution from coating the inside of the mouth. The mouth was then cleansed a second time by rinsing with water, and the patient remained seated during the remainder of the test. A breath sample was taken at 2 minutes in order to quantify urea hydrolysis in the oropharynx, and further samples were taken at 15, 20, 25, and 30 minutes.

Of the 154 patients, 104 were HP+ as determined by biopsy studies. Breath tests from the 49 HP− patients were used to define the negative (normal) range. For any time point, "normal" was a breath CPM value below the mean + 3SD obtained for the HP− group. A positive test was thus defined as a $^{14}CO_2$ excretion above this range.

In patients with *H. pylori*, the $^{14}CO_2$ excretion peaked about 10 minutes after ingestion of the isotope, then fell in an almost linear fashion. There was a 10-fold difference between HP+ and HP− patients (300 CPM vs 3000 CPM) (Fig. 12.10). At 15 and 20 minutes, the upper limits of normal for negative patients (mean + 3SD) were 1422 and 928 CPM, respectively. A scattergram of 20-minute samples from the 153 patients is shown in Fig. 12.11.

Improvements and theoretical limitations of the ^{14}C-urea breath test

Using a 5 μCi dose of ^{14}C-urea, we obtain CPM of less than 930 in HP− patients. To define a sample as 930 CPM ± 2.5% requires a maximum counting time of only 8 minutes. If the patient dose was decreased to 1 μCi, the upper limit of normal would be 200 CPM, which would require a counting time of up to 33 minutes for a borderline sample [69], but most samples could be defined as HP+ or HP− in less than 10 minutes. Collection of more than 1 mmol of breath CO_2 would allow further reduction of the isotope dose (more CPM collected), but we believe this is impractical insofar as it requires quite a prolonged breath collection.

Microdose version of the ^{14}C-urea breath test

Although 5 μCi ^{14}C-urea breath tests produce trivial radiation doses, a lower isotope dose is

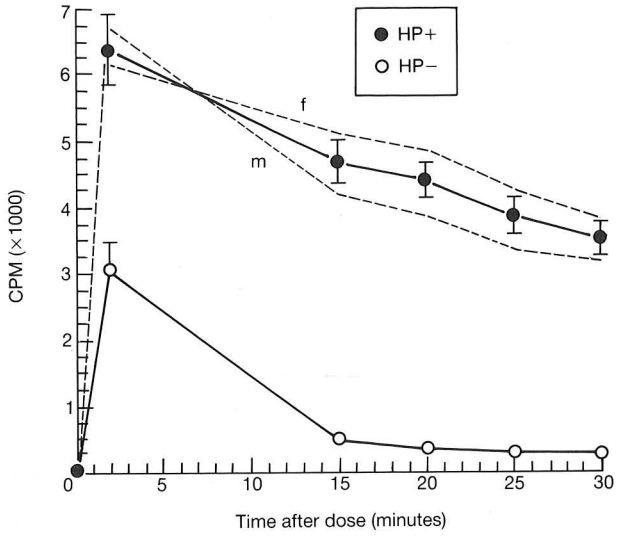


Fig. 12.10 Graph displaying the mean ^{14}C excretion between 2 and 30 minutes for the University of Virginia rapid 5 μCi breath test. In HP+ patients, females (f) gave slightly higher counts. At 15 and 20 minutes, upper limits of normal for negative patients (mean+3SD) were 1422 and 928 CPM, respectively. m, male patients.

desirable and theoretically possible. By supplying the isotope in a capsule form, ^{14}C-urea is not exposed to mouth urease. We have done preliminary evaluation of a microdose capsule-based breath-test methodology, with the aim of achieving optimal safety, speed, accuracy, and cost.

Individual 1 μCi doses of ^{14}C-urea were mixed with lactose and loaded into gelatin capsules. In preliminary studies ($n = 5$), dummy lactose capsules were given prior to routine endoscopy and observed endoscopically 5 minutes after ingestion. Thirteen patients biopsied for HP (seven HP+) fasted for 6 hours, then swallowed a capsule with 20 ml of water. Breath was collected in bags at 2, 4, 8, 12, 20, and 30 minutes. Samples were pumped through hyamine solution and read in a scintillation counter to an accuracy of ±2.5%. To assess reproducibility, HP+ patients repeated the test the following day.

Capsules viewed at endoscopy had all disintegrated on the corpus mucosa. In the breath test, HP+ patients averaged 1926 ± 578 CPM at 12 minutes, whereas HP− patients averaged 47 ± 11 CPM. Fig. 12.12 shows excretion curves for the HP+ group. At 12 minutes the test easily differentiated between patients as either HP+ or HP−. Correlation between first and second tests was also high ($r = 0.78$). Total radiation exposure was equal to environmental background received in 24 hours. Our initial assessment is that the microdose capsule-based ^{14}C-urea breath test is safe, fast, as accurate as earlier variations of the test, simple to perform, and environmentally benign (very little scintillation fluid is needed because patients may be diagnosed with a single breath sample taken at 15–20 minutes).

Safety of ^{14}C-urea

Biologic and physical half-life of ^{14}C

In the ^{14}C-urea breath test, ^{14}C-urea is either hydrolyzed and expired as $^{14}CO_2$, or excreted unchanged as urea in urine. Because the biologic half-life is so short (hours–days), the total cumulative radiation dose received from each breath test is small, and far below variations in natural radiation received within the United States. Concern with ^{14}C usually arises because of its long half-life, but this is immaterial for compounds such as CO_2 and urea, which are rapidly excreted.

Dose equivalence (DE)

To allow radiation sources to be compared, emissions of various origins are expressed in "dose equivalence (DE) units." The DE for ^{14}C takes into account the fraction of isotope

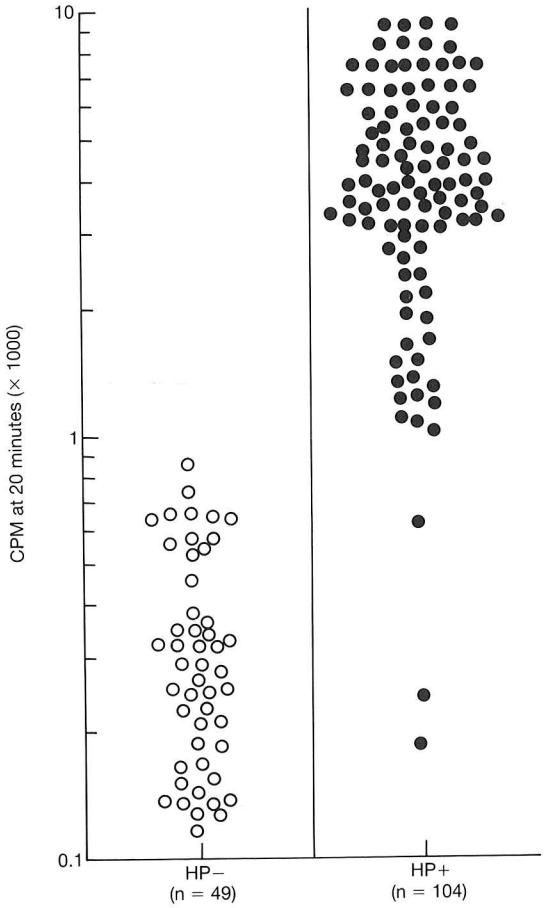


Fig. 12.11 ^{14}C-urea breath tests from 153 patients. With the logarithmic Y axis, large differences between HP− and HP+ are less apparent. (From Marshall *et al.* [66].)

sequestered in each organ, the biological half-life of the isotope in that tissue, the effective energy of β particles compared with standard ionizing radiation (a 200 kV X-ray source), and the administered dose. The long half-life of ^{14}C simplifies calculations, because the activity of the administered isotope can be regarded as constant for the time it is present in the body. DE is expressed in Sieverts (1 Sievert [Sv] = 100 rem, 10^{-5} Sv = 1 mrem).

Environmental sources of radiation

The average DE from cosmic radiation, terrestrial radiation, and endogenous naturally occurring radioisotopes in the United States is a minimum of 88 mrem year^{-1} [70]. In addition, the population is exposed to approximately 50 mrem year^{-1} of radiation due to diagnostic X-rays [71]. Therefore, DE for the average American is 2–3 mrem week^{-1} (much higher if recent observations of natural radon exposure in the United States are taken into account). Natural radiation is doubled for persons living in Colorado, and may also be increased by air travel at the rate of 0.3 mrem hour^{-1}. Because most people in the United States do not concern themselves with the risk of radiation from air travel or living in Colorado, radiation doses of less than 10 mrem year^{-1} are generally regarded as trivial. In addition, it should be noted that a normal person already carries ~0.1 μCi (3.7 kBq) of naturally occurring ^{14}C within body tissues.

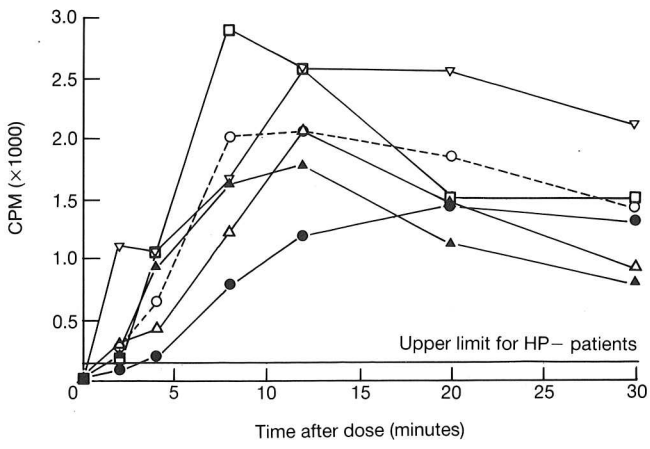


Fig. 12.12 Microdose capsule ^{14}C-urea breath test. Dose = 37 kBq.

Table 12.2 The ^{14}C-urea breath test compared with other common sources of radiation exposure

	185 kBq (5 μCi) ^{14}C-urea (mrem**)	Chest X-ray (mrem)	Upper GI series (mrem)	Natural sources* (2 weeks) (mrem)	Round trip by air, L.A.–N.Y. (mrem)
Bone	14.2	—	—	5.0	3.0
Breasts	3.5	14.0	53.0	5.0	3.0
Lungs	0.5	4.0	476.0	5.0–40.0***	3.0
Thyroid	0.3	6.5	7.0	5.0	3.0
Marrow	0.3	3.0	114.0	5.0	3.0
Testes	0.3	<0.01	0.4	5.0	3.0
Ovaries	0.3	0.06	45.0	5.0	3.0

Data from references [62, 70–76].
* Highest in Colorado (9 mrem); lowest in Louisiana (3 mrem).
** 1 mrem = 10^{-5} Sievert (Sv).
*** Lung doses much higher in high radon areas.

Calculations for ^{14}C-urea from existing data

The maximum radiation dose received from the ^{14}C-urea breath test occurs, in a theoretical subject, when 100% of the ingested urea is broken down by gastric urease and all the CO_2 enters the blood bicarbonate pool. From ^{14}C-labeled bicarbonate studies [72], it is known that in the first 5 hours, 75% is expired in the breath. The remaining 25% of the ^{14}C is taken up by metabolic processes with an excretion half-life of 10–12 days in soft tissues. Of this sequestered isotope, 10% (2.5% of the administered dose or 0.125 μCi in our test) enters bone and is excreted with a half-life of 40 days. Taking all deposited ^{14}C into account, the cumulative gonadal dose is a maximum of 0.06 mrem for every 1 μCi ^{14}C administered; i.e. 0.3 mrem in a 5 μCi breath test.

There is some concern about the DNA transmutation effect of ^{14}C. In brief, ^{14}C changes to nitrogen when it decomposes, an event which may cause mutation if it takes place within a DNA base pair. In experimental studies this effect is difficult to demonstrate, so that the transmutation effect of ^{14}C is not significantly more than the DE received from an equivalent γ-ray source [73]. A very conservative approach suggested by Totter *et al.* [74] is to double the effective DE for ^{14}C in order to account for any transmutation effects. It can be seen from Table 12.2 that natural radiation in 2 days gives more exposure to marrow and gonads than the 5 μCi ^{14}C-urea breath test [75], and 100 breath tests give less marrow exposure than a single upper gastrointestinal series (barium meal) [76].

Restrictions on use of the ^{14}C-urea breath test

The test should be used with similar guidelines to radiologic procedures. For example, ^{14}C-urea should not be given to women of childbearing potential. It is our practice to observe the 10-day rule and obtain a pregnancy test if there is doubt about the possibility of pregnancy.

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