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Mini Review

Helicobacter pylori urease for diagnosis of *Helicobacter pylori* infection: A mini review

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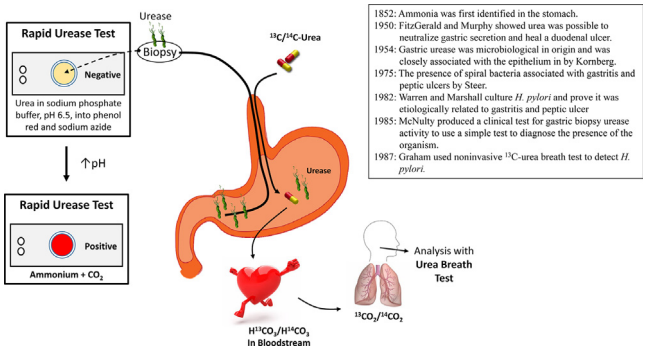
GRAPHICAL ABSTRACT



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ABSTRACT

The stomach contents contain of both acid and proteolytic enzymes. How the stomach digests food without damaging itself remained a topic of investigation for decades. One candidate was gastric urease, which neutralized acid by producing ammonia from urea diffusing from the blood and potentially could protect the stomach. Discovery that gastric urease was not mammalian resulted in a research hiatus until discovery that gastric urease was produce by *Helicobacter pylori* which caused gastritis, peptic ulcer and gastric cancer. Gastric urease allows the organism to colonize the acidic stomach and serves as a biomarker for the presence of *H. pylori*. Important clinical tests for *H. pylori*, the rapid urease test and urea breath test, are based on gastric urease. Rapid urease tests use gastric biopsies or mucus placed in a device containing urea and an indicator of pH change, typically phenol red. Urea breath tests measure the change in isotope enrichment of ^{13}C- or $^{14}CO_2$ in breath following oral administration of labeled urea. The urea breath test is non-invasive, convenient and accurate and the most widely used test for non-invasive test for detection of active *H. pylori* infection and for confirmation of cure after eradication therapy.

Introduction

Ammonia was first identified in the stomach in 1852 and since that time has remained a target of investigation [1]. Even today, medical devices designed to detect breath ammonia originally produced in the stomach are in use clinically to detect infection with the Gram negative bacterium, *Helicobacter pylori,* an important human pathogen that despite a decline in prevalence still infects approximately 50% of humans worldwide. *H. pylori* infection is the most common causative agent of gastritis, peptic ulcers and gastric cancer [2]. The presence of urease in the stomach was discovered early in the 20th century (reviewed in [1] and [3]).

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The discovery was followed by widespread interest in gastric urease including the range of animals in which urease could be found as well as its role in health and disease.

In the late 19th century, it was discovered that gastric cancer was somehow related to achlorhydria or loss of the stomach's ability to make acid [4]. This observation prompted research in gastric physiology and which was greatly heightened by the fact that at that time gastric cancer was the most common cause of fatal human cancers [5]. The late 19th century and the early 20th century was a time of great interest and research in gastric physiology and gastric disease [4,6]. That period was also the time many of the great gastrointestinal physiologists were making their discoveries. By 1900, gastric surgery had also begun to emerge as a new field especially devoted to peptic ulcer disease which was then often considered a surgical disease [4]. Duodenal ulcer, previously thought to be rare was found to actually be very common [4]. It was recognized that ulcers were somehow related to acid and that duodenal ulcers were associated with high acidity and gastric cancers with absence of acid.

Gastric urease

How the stomach protected itself from injury by the highly concentrated acid contained within was unclear and the object of considerable research [7,8]. For example, it was known that placing the leg of a live frog into the stomach through a hole in the abdominal wall would result in digestion of its flesh discounting the protective effect of a living principle [8]. Urea hydrolysis produced alkaline ammonia was thought to be a good candidate for the mechanism of protecting the gastric mucosa from the corrosive acid resent in the stomach [1]. FitzGerald and Murphy provided proof of principle that urea could play an important role in protecting the stomach by showing that it was possible to neutralize gastric secretion and heal a duodenal ulcer by giving urea orally and parenterally to humans [9].

Much of the credit for our current understanding gastric urease comes from decades of experiments by Kornberg et al. who studied gastric urease primarily in cats [3,10]. Their comprehensive studies have served as the basis for modern investigations. The breadth of Kornberg's observations included studies on: (*a*) the effect of acid secretion on urea breakdown, (*b*) the effect of the presence of acid in the stomach, variations in gastric blood flow, and the secretion of non-acid juice on urea hydrolysis, (*c*) urea hydrolysis associated with the passage of urea solution from the gastric lumen to the blood, (*d*) the effect of anti-bacterial substances on urea hydrolysis, (*e*) the deposition of urease in the stomach, (*f*) urea and ammonia content of gastric juice, (*g*) quantitative aspects of gastric urease activity, and (*h*) disappearance of urea from the gastric juice. Their conclusions, regarding the physiology of urea in the stomach, included: (*a*) hydrolysis of urea was associated with its passage from blood in both parietal and non-parietal secretions to the lumen of the stomach, (*b*) the amount of urea hydrolyzed paralleled the rate of secretion of acid juice, (*c*) when urea was added to the stomach, the rate of urea hydrolysis was determined by the rate of passage of urea-containing fluid through the mucosa; the majority of the urea was hydrolyzed in the mucosa before entering the blood, and finally and most importantly, (*d*) gastric urease was microbiological in origin and was closely associated with the epithelium [1,3,10]. The evidence suggesting a microbial origin of urease initially rested on the effects of treatment with antimicrobials, for example, cats with gastric urease activity had a mean gastric juice urea and ammonium concentration of 0.6 mM and 4.2 mM respectively. Following antimicrobial therapy, the mean concentrations of urea rose and that of ammonium fell (3 mM and <0.05 mM, respectively) [3,10].

Overall, interest in gastric urease waned after it was shown that urease was not a mammalian enzyme but rather was likely of microbial origin [1,3]. Interest was rekindled by the discovery of *H. pylori* in 1982 and its role in gastritis and peptic ulcer disease [11]. After the discovery of *H. pylori* interest in the role of urease in human disease was rekindled including the role of gastric urease in relation to the production of ammonia which had a role in the neurologic complications of liver disease ranging from hepatic encephalopathy to hepatic coma [12–18].

The mid-20th century was an era characterized by great interest in peptic ulcer disease. For example, during the 1970s there were more than 140,000 ulcer operations/year in the United States [4] and Congress established specific research centers to solve the ulcer problem known as Centers of Ulcer Research and Education (CURE) [19]. It was known that duodenal ulcer was associated with specific abnormalities in the control of gastric secretion and this guided CURE's research. The hypothesis of a microbial cause of peptic ulcer had gone in and out of favor for decades. In the mid-1970s a group in the UK noted the presence of spiral bacteria associated with gastritis and peptic ulcers [20–22]. Attempts to culture that organism failed. Later, Robin Warren from Australia prompted by his observations using silver stained gastric sections, also noted the association of spiral bacteria and gastric inflammation. He was able to convince a clinical research fellow, Berry Marshall, to join him and together they were able to confirm their observations and with the advice of a microbiologist, Adrian Lee, who had special expertise with spiral bacteria, and the laboratory services of Steward Goodwin were able to culture *H. pylori* and prove it was etiologically related to gastritis and peptic ulcer. For this, Warren and Marshall earned a Nobel Prize in 2005 [23].

H. pylori urease

Although initial microbiological studies pointed away from *H. pylori* being urease positive, subsequent studies by McNulty et al. reported copious urease activity [24–26]. McNulty et al. also produced a clinical test for gastric biopsy urease activity to use a simple test to diagnose the presence of the organism [24,26]. Marshall subsequently added an antimicrobial agent to a common laboratory urease test and produced the first patented test to detect *H. pylori* clinically, the CLO test for *Campylobacter*-like organisms as *H. pylori* was then called. *H. pylori* urease also proved to be highly antigenic and is a component of most anti-*H. pylori* serologic tests and candidate vaccines.

The tolerance of *H. pylori* to acid is largely dependent on urease activity, a cytoplasmic enzyme. Access of urea to the enzyme is restricted by the presence of a H^+-gated pore (UreI) such that in acidic conditions urea can enter the cytoplasmic space and be hydrolyzed to CO_2 and ammonia [27–30]. The gene product is directly responsible for urea permeability and is active at acidic pH or it regulates the urea permeability of another cytoplasmic membrane protein. The ammonia produced diffuses into the low pH stomach where it becomes ionized and trapped in the gastric lumen whereas the CO_2 appears in the blood and subsequently is exhaled.

During the Kornberg era ^{14}C and ^{13}C isotope-based methodology was used to identify urease activity non-invasively as quasi-breath tests in experimental animals including the frog [1,3]. Methods using stable and radioactive isotopes were subsequently used to develop diagnostic tests in humans utilizing isotopic enrichment of breath, blood, or urine following oral administration of labeled compounds, most often urea, to detect the presence of *H. pylori* infections [31–37]. Although urea and ammonia can easily be measured in gastric juice [38], the first clinically useful rapid tests for diagnosis of *H. pylori* using gastric contents or biopsies targeted urease and were adaptations of standard laboratory tests for urease activity and named Rapid Urease Tests (RUTs).

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