*Helicobacter pylori: Physiology and Genetics*
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Chapter 3

# One Hundred Years of Discovery and Rediscovery of *Helicobacter pylori* and Its Association with Peptic Ulcer Disease

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Today's understanding of *Helicobacter*-related gastric diseases in humans stems from an explosion in research, which occurred after the first culture of the organism by Marshall and Warren in 1982 (47). This event may have been the culmination of over 100 years of study of helicobacters and their epiphenomena. This chapter is a concise overview of major highlights during that time following four storylines of spiral bacteria, epidemic gastritis with hypochlorhydria, urease, and bismuth therapy.

## SPIRAL BACTERIA

The first well-known report of gastric helicobacters was by Bizzozero in Turin in 1893 (1). Bizzozero was a well-known anatomist, famous already for his proof that all dividing cells required cell nuclei (4). In his anatomical observations of the gastric mucosa of dogs, Bizzozero reported "spirochetes" inhabiting the gastric glands (9) and even the canaliculi of the parietal cells. In hand-drawn color illustrations, Bizzozero showed gram-negative organisms with approximately 10 wavelengths within the parietal cells and gastric glands. We now know these organisms variously identified as *Helicobacter canis, Helicobacter felis* (24), and/or *Helicobacter heilmannii* (18). Bizzozero's work was extended by Salomon, who was able to propagate these spiral organisms in mouse stomachs after feeding ground-up gastric mucosa from cats and dogs to his mouse colony (42). Salomon's work was a precursor to current studies where the *H. felis*-infected mouse is an important model in vaccine and therapeutic studies of helicobacter eradication (5).

In the 20th century, anatomists and pathologists noticed spiral organisms in the human mucosa from time to time, initially adjacent to carcinomas (21). The organisms were noted in the gastric mucosa of macaques by Doenges in the United States (7), and in resected gastric specimens by Freedberg and Baron in 1940 (11). Freedberg and Baron found "spirochetes" in about 40% of resected specimens. In retrospect, the population of the United States was probably more than 40% infected with *Helicobacter pylori* at that time, but because patients undergoing gastric surgery had such severe disturbances of their physiology, the helicobacters such as *H. pylori* may have actually regressed or disappeared, as is believed to happen in late gastric carcinoma cases today. Nevertheless, Freedberg and Baron presented their findings in 1940 and generated a vigorous discussion in which some members of the audience, used to treating syphilis with heavy metals such as mercury, arsenic, and bismuth, commented on anecdotal cases of complete remission of peptic ulcer disease while treating the syphilis spirochetes.

Doenges and Freedberg were challenged in the early 1950s by Palmer, who found no evidence of spirochetes in more than 1,000 gastric biopsies taken with a blind suction biopsy instrument (37). To this day, no one knows how such an incorrect conclusion could have been made at a time when more than 50% of the population was bound to be positive for *H. pylori*. One can only guess that the appropriate stains were not performed or that the investigators were looking for something other than *H. pylori* and discounted the presence of black, silver-stained prolific organisms.

In the 1950s and 1960s, Susumu Ito of Harvard Medical School made some of the first detailed anatomic descriptions of the gastric mucosa appearance under the electron microscope. Ito's photographs and drawings of the structure of the parietal cell and acid-

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secreting glands of the gastric corpus are well known and have been copied in many medical texts. However, he also observed "spirilli" in some of his material. He published an excellent photograph of one of these organisms in 1967, showing a greatly enlarged *H. pylori* within a parietal cell gland, complete with several sheathed flagella and typical spiral morphology (20). In subsequent years, Lockard and Bolar found these organisms once more in the stomach of cats and dogs and also published electron micrographs of them (25).

In the mid-1970s, the spiral bacteria were the subject of a paper by Steer and Colin-Jones (43). Their research occurred at the threshold between old and new therapies for peptic ulcer, i.e., carbenoxolone or bismuth versus the new drug, cimetidine. Steer and Colin-Jones studied the presence of bacteria and inflammation during gastric ulcer healing with carbenoxolone. They found that the drug healed the ulcers but had no major effect on the histology, because the inflammation was just as severe after ulcer healing. They noted that numerous spiral bacteria were present in 80% of their gastric ulcer specimens. Unfortunately, they were unable to culture the organism, or, at least, only cultured pseudomonas. It was several more years before the microaerophilic campylobacter isolation techniques were well known, so their mistake is understandable. Nevertheless, they published excellent photographs of the gastric mucosal histology, including *H. pylori* in the mucous layer and even phagocytosed within neutrophils. Further papers followed, and some of the best illustrations of *H. pylori* on duodenal mucosa at ulcer borders subsequently appeared in a publication in *Gut* a year or so after the Australian culture and rediscovery of the organism (44).

The interest of pathologists and anatomists in the spiral bacterium remained rather focused, however, and divorced from clinical observations on gastric diseases. For this reason, persons studying gastritis and clinicians studying peptic ulcer disease were generally unaware of the spirochete literature, and never considered bacterial causes of gastric diseases. Nevertheless, after *H. pylori* was cultured and observed to be present in so many people, both symptomatic and healthy, the question was asked: where did these organisms arrive from, and what happened during the acute infection with helicobacter?

## EPIDEMIC GASTRITIS WITH HYPOCHLORHYDRIA

As with the spiral organism literature, the medical literature from the late 19th century contains some of the answers. In their *Principles and Practice of Medicine* volumes published between 1900 and 1920, Osler and McCrae described an acute form of gastritis with hypochlorhydria (36). They were not the first to observe this syndrome, since this had been reported in several earlier medical texts. Because endoscopy was not possible, the exact pathophysiology of this disorder was unknown, but it was known to be a common syndrome in young children, characterized by a transient vomiting illness with neutral pH of the gastric fluids. Various treatments were advised, including bismuth, and the occasional patient who developed a chronic dyspeptic syndrome was described. This epidemic-gastritis-with-hypochlorhydria syndrome remained in the medical textbooks until about the mid-1960s, when its rarity led to its deletion from most chapters on gastritis or gastroenterology. It was, therefore, a surprise when investigators began encountering epidemics of hypochlorhydria in the 1970s when it again became popular to study gastric acid secretion by passing nasogastric tubes in volunteers (39). Basil Hirschowitz reported this illness from Birmingham, Ala., where acute mucosal changes were thought to be due to the effect of cortisone on the gastric mucosa (19). More recently, an epidemic was reported by Richard Hunt that occurred in several volunteers undergoing gastric analysis in the British Navy (13). The most notable of these reports came from the laboratory of Fordtran, where Ramsay and colleagues noticed that more than half of their volunteers undergoing gastric analysis developed hypochlorhydria associated with gastritis (39). The hypochlorhydria lasted several months and was associated initially with a vomiting illness and even abnormal liver function tests in a few individuals. In retrospect, this was acute *H. pylori* infection transmitted from one volunteer to the next by the nasogastric tubes or by contamination within the laboratory itself. Reexamination of these individuals more than 15 years after the event revealed that they still had *H. pylori*, but few had developed peptic ulcer disease; in fact, most of them were asymptomatic (17). One other notable episode reported by Wiersinga and Tytgat in Holland (49) occurred in a patient with Zollinger-Ellison syndrome who developed acute gastritis after endoscopy and subsequently was hypochlorhydric. Retrospective examination of those sections revealed *H. pylori* from what was probably a case of endoscope-transmitted *H. pylori*. Wiersinga's observations are of interest because he suggested that the infectious agent, whatever it might be, could be used as therapy for patients with Zollinger-Ellison syndrome.

Thus, the acute syndrome of *H. pylori* had been well described in the literature before the presence of the organism in the hypochlorhydric syndrome was

noted in the 1980s. Further studies in this vein continue, so that even volunteer experiments have been described (28, 34) as well as sporadic accidental reports of laboratory-acquired infections (32, 46), all confirming the earlier observations.

## THE ORIGIN OF GASTRIC UREASE

Without regard to the spirochete and hypochlorhydria literature, other scientists studied gastric urease enzyme from the 1920s. Urease was an important enzyme because it was the first enzyme purified into crystalline form whereby analysis could identify that it was actually a protein. Prior to Sumner's observations on jackbean urease (45), the exact composition of enzymes was not known, and whether they were proteins or merely cofactors was highly controversial. In 1924, Murray Luck and colleagues studied the urease enzyme of the gastric mucosa of dogs (26). They observed that many carnivorous animals, particularly cats and dogs, had high levels of gastric urease present. They equated strength of the enzyme with that present in raw jackbeans (*Canavalia ensiformis*), i.e., a very high amount. Since Luck could not separate the urease from the gastric mucosa, he presumed it was intrinsic to the epithelial cells and was secreted into the mucous layer. The purpose of this urease was unknown, but he postulated that it acted as a safety valve, whereby animals with uremia could destroy excess blood urea with gastric urease and vomit up the ammonia-laden secretions. Thus, uremic vomiting could be explained by the presence of the urease enzyme.

Gastric urease in humans was the subject of a thesis by Fitzgerald and Murphy published in the *Irish Journal of Medical Science* in 1950 (10). They could not perform endoscopy, however, so most of the specimens again came from patients who were having partial gastrectomies for peptic ulcer disease or gastric cancer. Thus, almost all gastric specimens had urease present. Fitzgerald and Murphy went a step further, measuring urea levels in the gastric juice and proposing that urea could be used as an antacid, since it was broken down into alkaline, ammonia, and bicarbonate. Again, Fitzgerald made the mistake of assuming the urease was part of the gastric mucosa, probably because he had inadequate control material. Fitzgerald's research resulted in trials of urea in patients with refractory peptic ulcer disease, but the amounts of urea required to be taken by the patients were in the range of several grams and tended to cause severe side effects such as vomiting.

A milestone in the study of gastric urease came in the late 1950s when Charles Lieber and his colleague Lefevre in Belgium and subsequently in New York studied the gastric urease of alcoholics. The interest was that this urease could contribute to blood ammonia levels and therefore induce hepatic encephalopathy in persons with cirrhosis. They measured the gastric urea and ammonia concentrations by aspirating gastric juice from their subjects and showed that tetracycline caused reversal of the normal ratio. That is, whereas "normal" persons had urea nitrogen present mainly as ammonia, treatment with antibiotics resulted in urea nitrogen being present in gastric juice, mainly as urea. Thus, they had evidence for a bacterial origin of gastric urease and justification for the treatment of encephalopathic persons with antibiotics, including neomycin, kanamycin, and other antibiotics. Their work was largely forgotten, because subsequent studies of hepatic encephalopathy and antibiotic use focused mainly on the presence of urease in the colon. Gastric urease remained a little-studied phenomenon until the high urease production of *H. pylori* and other helicobacters was observed first by Langenberg (23). This and the observations of others led to the widespread use of the currently available rapid urease tests (e.g., the CLOtest 31), a simple diagnostic for detecting *H. pylori* by showing presence of its urease enzyme in gastric tissue.

Knowledge of gastric urease and observations on the urea concentration in gastric juice by Marshall and Langton in 1984 (29) led to the development of the urea breath test, a noninvasive form of detecting gastric urease (14, 30).

## BISMUTH SALTS FOR GASTRIC DISEASE

The final thread tracking the history of *H. pylori* is that associated with bismuth use. Bismuth compounds were used in ancient times, along with other heavy metals, as an antiseptic and cosmetic. From the 17th century, bismuth, antimony, and mercury were used in various concoctions, usually to induce purging. In retrospect, prior to the 20th century, almost all individuals were infected with *H. pylori* (2), so it was only a matter of time before a patient with severe gastric symptoms underwent sudden remission after being treated with large doses of oral heavy metal salts. As a result, by the 19th century, bismuth compounds were advocated in the form of bismuth subnitrate, subcarbonate, and subcitrate for the treatment of nonspecific gastrointestinal symptoms. Ogle described "the fruit acid of bismuth" for "nervous disorders of the bowels" (35). His formulation may have been the precursor to the modern drugs De-Nol and ranitidine bismuth citrate. In the United States, a patent medicine called Bismosal was devised by the Nor-

wich Company. Bismosal was a suspension of bismuth subsalicylate, which, unlike some other salts of bismuth, for example, the subnitrate, was relatively insoluble and had low toxicity. Bismosal was used for treating infantile cholera (probably *Campylobacter jejuni* infection) and, according to the packet insert, was very useful for patients with gastritis. Subsequent experiments confirmed that bismuth was extremely toxic to helicobacter and campylobacter species, and also had inhibitory effects on many other gut organisms (8, 27). As well as bismuth, 19th-century physicians used other exotic metals from time to time for severe gastric disturbance, even going so far as silver nitrate gastric lavage for the treatment of refractory gastritis.

Whereas German antacid formulations continued to contain some bismuth, usually in the form of bismuth subnitrate and bismuth subcarbonate, until modern times, American formulations of antacids were without bismuth after 1950. This deliberate omission resulted from the work of Ivy, who observed that these salts were quite poor buffers and therefore contributed very little to the antacid effect. Nevertheless, the grandson of Bismosal, Pepto-Bismol, gradually came into wide use as an over-the-counter medication for the treatment of nonspecific episodes of vomiting and diarrhea in the United States. It is interesting to note that double-blind clinical trials of ulcer relapse in the United States always saw far fewer relapses than similar studies performed in Europe. Since antibiotics and Pepto-Bismol were never restricted in U.S. studies, it is quite possible that many of the ulcer patients in both the active and placebo groups were freely taking doses of Pepto-Bismol during their follow-up period. In the mid-1970s, the Gist-Brocades Company of Holland, which manufactured and sold bismuth subcitrate solution at that time, developed a tablet form that was far more palatable and, therefore, could be widely marketed. However, by that time, bismuth had been banned in France because of chronic overdose and heavy metal toxicity in thousands of people in the 1950s. However, in countries that allowed bismuth use, the drug De-Nol demonstrated a lower ulcer relapse rate than was seen in patients who merely had acid reduction therapy with H2 blockers such as cimetidine. The low relapse rate, or even absence of relapse in one-third of patients taking the bismuth treatment, suggested that the ulcer problem had been totally cured in these persons. Warren and Marshall took this as supportive evidence toward the hypothesis that *H. pylori* had been eradicated in these persons and that the organism was responsible for the ulcer in the first place. Further evidence of this was obtained in publications by Gregory, Moshal, and Spitaels (16) showing the improved healing of duodenal ulcer borders after treatment with bismuth versus treatment with H2 blockers. In their illustrations of the electron micrographic appearance of these duodenal ulcer borders, Gregory and colleagues showed numerous helicobacters in the pretreatment patients, but not in the posttreatment patients, again supporting the developing hypothesis.

During the 1980s, as the links between *H. pylori* urease, epidemic gastritis, and peptic ulcer were becoming recognized, investigators tried to explain some of the more subtle features of peptic ulcer disease on the basis of the helicobacter infection. The most important of these may have been that gastritis, by suppressing D-cell function and somatostatin release in the antral mucosa, could lead to lifelong mild hypergastrinemia in some persons, thus explaining the shift of the normal acid secretion distribution to the right in populations of patients with peptic ulcer disease (12, 38). The supposed "hereditary" predisposition of some families for peptic ulcer, previously explained on the basis of stress and inherited manifestations such as elevated serum pepsinogens (41), could now also be explained by *H. pylori,* since serum pepsinogens reflected inflammation in the gastric mucosa and reduced once the bacterium had been eradicated.

## SYNTHESIS: WARREN AND MARSHALL 1979–84

The observations of Warren and Marshall between 1979 and 1984 allowed these and other investigators to tie together the various threads that had been constructed in the medical literature in the preceding 100 years. Warren had observed patients with spiral organisms on their gastric mucosa since 1979 and had documented the inflammation associated with the bacteria by the time he and Marshall began a concerted attempt to study the organisms in patients with various upper gastrointestinal symptoms. After August 1981, the team studied patients attending for endoscopy and were able to demonstrate the gram-negative bacteria on Gram stains but could not culture them at that time. They tentatively treated one patient with tetracycline and were able to observe a decrease in the number of neutrophils in the gastric mucosa as well as apparent disappearance of the bacteria. They recognized, however, that anecdotal evidence of the bacteria's role in gastric inflammation was of little value and therefore commenced a study in 100 consecutive endoscopy patients to try to culture the bacteria, as well as determine their association with gastritis and/or other clinical syndromes. Initially, they did not focus specifically on the etiology of peptic ulcer disease, although they were aware that gastritis was strongly associated with duodenal and gastric ulcers,

as well as with gastric cancer (47). Thus, in the beginning of 1982, Marshall and Warren commenced a study whereby patients attending for endoscopy were biopsied, after signing the appropriate consent and completing a questionnaire detailing dental hygiene, dietary habits, nonsteroidal anti-inflammatory drug and antacid use, etc. Only two biopsies were taken from the antrum: one was for culture using a variety of techniques but mainly microaerobic incubation similar to that used for campylobacter; the second biospsy was sent for histological examination by Dr. Warren using hematoxylin and eosin and Warthin-Starry silver stains. It was not until patient 35 was biopsied that the organism was grown. That event came about because of a lucky accident, in which the cultures were left in the incubator over the long Easter weekend and thus the plates were not examined until the fourth or fifth day after biopsy. When the water-spray 1 mm transparent colonies were observed, the technologist realized in hindsight that, prior to this day, the research biopsies had been discarded after 48 h when normal gastrointestinal or throat specimens would have been expected to be overgrown with commensal flora and thus would have been useless for any further diagnostic purpose. This rule did not apply to *H. pylori* cultures, however, because they were from clean gastric biopsy specimens and, in most cases, there were few throat commensals on the nonselective blood agar plates. After that time, *H. pylori* could be grown using microaerobic techniques on blood or agar plates with relative ease in Australia. The organism was first cultured outside Australia in September 1983 by McNulty and Skirrow from a gastric ulcer patient in Worchester, England (33).

In the initial 100 patients studied by Marshall and Warren, more than 65% of patients were infected with the organism, and almost all of them had gastritis ($P < 0.000001$). Of particular interest to the investigators, however, was the fact that all 13 patients with duodenal ulcer in that study were found to have the organism, and 18 of 22 gastric ulcer patients also had the organism. Ulcer patients without *H. pylori* tended to be taking nonsteroidal anti-inflammatory drugs. This finding accelerated interest in the organism among gastroenterologists, and the results were confirmed in several countries within a year or so (22, 33). Double-blind trials had limited success in the ensuing 5 years because the eradication rates of the available therapies (bismuth with one antibiotic, for example) were rather low, so that in a large study, only about one-quarter of treated patients actually achieved *H. pylori* eradication. Nevertheless, Coghlan et al. (6), Rauws and Tytgat (40), and Graham et al. (15) confirmed that *H. pylori* eradication cured peptic ulcer.

## QUESTIONS FOR THE FUTURE

Now that the *H. pylori* link with peptic ulcer and the role of antibiotic therapy in this disease have been accepted, therapies have advanced to the point that almost all patients can be eradicated of the bacteria with antibiotic combinations that include an acid-lowering or bismuth component. Associated diseases such as gastric cancer, and even gastric lymphoma, continue to excite interest, and Koch's postulates have been fulfilled for peptic ulcer and gastric cancer in an animal model (the Mongolian gerbil) (48). Nevertheless, controversy still reigns over *H. pylori*. Is it always a pathogen, or is it sometimes a commensal? Is there a beneficial role for the organism in some persons or populations, as has been suggested by Blaser and others (3)? If *H. pylori* is a harmful pathogen, how harmful? Is it cost-effective to eradicate all *H. pylori* or just toxin producers? These and other interesting questions are addressed by other authors in this volume.

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