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History of the Discovery of *C. pylori*

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EARLY OBSERVATIONS OF "GASTRIC SPIROCHAETES"

Gastric spiral bacteria were described by Bizzozero before the turn of the century.[1] His studies were followed by those of Salomon[2] who reported these "spirochaetes" in the stomachs of numerous adult animals, usually carnivores. The first observation of such organisms in the human stomach may have been by Kreinitz.[3] When gastric spiral bacteria were first discovered, microbiology itself was an infant science and gastric infections were low on a long list of infectious disorders to be tackled.

At that time, no antibiotics were available, and antibacterial therapy was restricted to antiseptics and heavy metals. The most well known were the arsenicals, but elements in the same chemical group such as antimony and bismuth were also popular therapies, particularly for syphilis.[4] In addition to its known antibacterial uses, bismuth had been used since 1800 as a remedy for epigastric discomfort and peptic ulceration.[5] Bismuth salts were said to have an astringent effect, meaning that they had the ability to precipitate proteins, arrest discharges, and contract tissues.[6] Although identical bismuth compounds were effective in syphilis, a possible connection between gastric diseases, gastric spirochetes, and ulcer therapy was overlooked.

Meanwhile, basic scientists interested in urea metabolism were unknowingly studying gastric spiral bacteria. In the 1920s, Luck[7] published extensively on the distribution of gastric mucosal urease in animal species. He could not separate urease from the epithelial cells, so he decided that the enzyme was an integral part of normal mucus secretion. In 1920 the urea cycle of Krebs[8] had not been discovered, and the urease of gastric mucosa was thought to be a mechanism for mammals to break down urea and excrete unwanted ammonia in the vomitus.

SPIRAL ORGANISMS—COMMON IN HUMAN GASTRIC TISSUE

Ten years later, Doenges[9] studied the gastric spirochetes of macaques, and later described similar organisms, associated with gastritis, in postmortem human stomachs. Freedberg and Baron[10] searched for bacteria in resected stomachs and found the spiral organisms in 37%, proving that they were not merely the result of postmortem contamination. Freedberg recalls that he did not see the large numbers of organisms in his series that we find in the gastric mucosal specimens now taken from patients at endoscopy (personal communication, August, 1983). This may have been because of poor fixation in his specimens or, alternatively, antibacterial effects of "bowel preparation," which was commonly practiced before enteric surgery of the time. Freedburg described three types of spiral organisms. This appearance of multiple types may have been caused by the presence of unseparated dividing *C. pylori* organisms and/or coccoidal forms.

No further studies of the unknown organisms were done until Palmer[11] searched for them in a series of over 1,000 gastric biopsy specimens obtained at Walter Reed Hospital. Mysteriously, no spiral organisms were identified in that series and he mistakenly reversed the conclusions of Freedberg, deciding that the organisms were, after all, mere postmortem contaminants. Freedberg should perhaps be criticized for not fighting back and proving their existence. Now Professor Emeritus at Harvard Medical School in Boston, he describes himself as a jack-of-all-trades in research, and by 1954 he had become involved in several other fruitful research areas (personal communication, August 1987).

UREASE—THE MISSING CLUE

In 1950, a thesis on gastric urease and urea therapy for peptic ulcer disease was written by Fitzgerald and Murphy.[12] Fitzgerald later became president of the British Society of Gastroenterology. He died in 1987, but survived long enough to see a considerable number of Irish publications on *C. pylori*. Fitzgerald and Murphy concluded that urease was produced by the gastric mucosa as a mucosal defense against acid that penetrated the mucus layer. They found urease in nearly all of their human gastric specimens, although these were limited to resected stomachs. Because most gastric surgery was performed in response to peptic ulcer disease, we now know that nearly all would have contained *C. pylori*.

The gastric mucosa seemed to have remarkable urease activity, so it was studied further as a source of ammonia in persons with hepatic encephalopathy. Summerskill et al[13] treated encephalopathic patients with urease to see if gastric ammonia was an aggravating factor in encephalopathy. In a small study, no obvious effect was observed. In retrospect, both the treated and control groups would have included patients with *C. pylori*, an uncontrolled variable that may have ensured a negative outcome for the study. Subsequently, gastric urease was suggested to be bacterial in origin by Lieber and LeFevre,[14] who showed that it disappeared when patients were treated with tetracycline. The data tables from Lieber and LeFevre's paper show that not all patients in the study had gastric urease. Baseline gastric juice urea levels were low in some patients and

high in others.[15] As with other literature that might have provided clues to the existence of *C. pylori*, the significance of Lieber and LeFevre's work went unrecognized by numerous investigators who continued to argue about the origin of gastric urease. After 1960, effective therapy of hepatic encephalopathy with nonabsorbable antibiotics and lactulose allowed gastroenterologists to again forget gastric urease and teach that urea hydrolysis occurred primarily in the colon. In retrospect, many *C. pylori* infections may have been successfully treated during neomycin therapy for hepatic encephalopathy.

Surprisingly, after so many had tried to discover the origin and significance of gastric urease, virtually no work was done in that area after 1970. In 1968 Delluva[16] confirmed that there was no urease in (germ-free) fetal animals, therefore the enzyme was not a component of gastric tissue but likely related to colonization with bacteria. As far as we know, none of the urease investigators examined histologic sections of the gastric tissue they were studying.

HYPOCHLORHYDRIC GASTRITIS

Medical text descriptions of gastritis, particularly the acute syndrome, were other unrecognized early discoveries of *C. pylori*. Osler and McCrae[5] described a syndrome of acute gastritis and hypochlorhydria. In their words:

> Microscopically, the changes are chiefly noticeable in the mucous and peptic cells, which are swollen and more granular, and there is an infiltration of the intertubular tissue with leukocytes. . . . In severer forms the attack may set in with a chill and febrile reaction, in which the temperature rises to 102°F or 103°F. The tongue is furred, the breath heavy, and vomiting is frequent. . . . The abdomen may be slightly distended and somewhat tender in the epigastric region. . . . The attack may last from one to three days, and occasionally longer. The examination of the vomitus shows, as a rule, absence of hydrochloric acid, the presence of lactic and fatty acids, and marked increase in the mucus.

Osler's description and the subsequent chapters on acute and chronic gastritis imply that an acute illness associated with vomiting of slimy mucus was not uncommon in his day. In addition, it appears that it was routine to test the vomitus for the presence of hydrochloric acid, and that lactic and fatty acids sometimes also would be present. His description is compatible with the syndrome we call epidemic gastritis with hypochlorhydria, which later was shown by two self-administration experiments to be due to *C. pylori*.[17,18] Hypochlorhydria allows gastric colonization with aerobic and anaerobic bacteria and contamination of the gastric juice with their metabolic products, acetic and short chain fatty acids. The syndrome remained in the medical texts until the mid-1960s.[19] After that time, perhaps because it was no longer being seen, it was omitted. Although many investigators would have seen patients with achlorhydria during routine acid secretion studies, they were unaware of the syndrome described by Osler and rarely performed gastric biopsy.

As a result, only a few investigators observed the association between acute histologic gastritis and hypochlorhydria. The best examples were papers published in 1975 by Wiersinga and Tytgat[20] and later the epidemic reported from Dallas by Ramsey et al[21] in 1979. These are described in detail by Morris in

Chapter 5. I wrote to these authors in 1984 and asked to see the histologic sections. In each case, the sections contained *C. pylori*, as do nearly all mucosal biopsies showing active, chronic gastritis.

REDISCOVERY OF GASTRIC SPIRALS

In the 1970s, Steer and Colin-Jones[22] tried to revive interest in the gastric spiral bacteria. In a study of 50 stomachs resected for gastric ulcer, they reported the presence of gastric bacteria and gastritis in 80%. In the early 1970s, *Campylobacter* isolation using microaerobic incubation methods was unknown in most hospitals; therefore, *C. pylori* could not be cultured from Steer and Colin-Jones's specimens. Instead, *Pseudomonas* spp. were cultured and mistakenly identified as the gastric spiral bacteria. The error was due to a number of unlucky events, one of which must have been the section chosen for the electron micrograph in Steer and Colin-Jones's paper. They chose a transverse section of a single organism for the main illustration. Nearly all bacteria appear as circles if cut in transverse section. A longitudinal section might have allowed others to point out to Steer and Colin-Jones that the spiral, or "S" shaped, organism was not *Pseudomonas*. Steer tenaciously studied the gastric spiral bacteria for ten years and published several papers on them. He documented the association with gastritis[23] and the tendency of the organisms to adhere to metaplastic gastric epithelium in the duodenum of ulcer patients.[24]

THE FOREST AND THE TREES!

Indirectly, others were observing the gastric spiral bacteria and dismissing them as irrelevant contaminants. One of the best examples of a probable *C. pylori* organism was shown in Heidel and Code's *Handbook of Physiology*.[25] Ito, in a chapter on gastric mucosal structure and ultrastructure, reserved a full page for an illustration of a "gastric spirillum." The organism was cut in longitudinal sections and two sheathed flagella could be seen emerging from one end. At the Royal Perth Hospital in Western Australia, Fung et al[26] studied a series of patients undergoing endoscopy to see if they could correlate the endoscopic appearance with the gastric mucosal histology (they could not). In illustrations from their paper, curved bacteria were noted with the letter "B" and an arrow. Even better illustrations of the organism were present in a paper by Gregory et al.[27] Studying duodenal ulcer borders, they included seven bacterial profiles in one section, overlying an area of metaplastic gastric epithelium in a duodenal ulcer border.

WESTERN AUSTRALIA—FIRST OBSERVATIONS

Investigation of the gastric spiral bacteria themselves began in Australia in 1979 when Robin Warren, a pathologist at Royal Perth Hospital, Western Australia, observed them on histologic sections from patients with gastritis. On hematoxylin and eosin sections of an antral biopsy specimen from a middle-aged man with nonulcer dyspepsia, Warren saw severe active chronic gastritis and noticed what were probably microorganisms coating the mucosa. To see the bacterial

morphology more clearly he requested a silver stain and was impressed by the enormous number of curved and spiral organisms present.

Over the next two years Warren routinely requested Warthin Starry silver stains on gastric biopsies and observed the bacteria in many, usually in association with gastritis. He observed three components of the histologic pattern associated with the bacteria: epithelial cell damage, neutrophil infiltration (activity), and increased numbers of mononuclear cells (chronicity). This appearance was known in the histology texts as "active chronic gastritis".[28]

In 1981 I was in my second year of a three-year internal medicine training program and for six months was assigned to gastroenterology. Trainees were encouraged to carry out some clinical research and it was suggested to me by the chief of gastroenterology, Tom Waters, that I help Warren investigate the bacteria he had observed.

THE PROBLEM OF GASTRIC SPIRAL BACTERIA: JULY 1981

When I commenced studies into the gastric spiral bacteria, Warren had already observed the association with gastritis, but the data were difficult to interpret because not all patients undergoing endoscopy were biopsied. First, Warren's sections were a selection from persons with endoscopic abnormalities, particularly gastric ulcers. Therefore, the histologic observations could not easily be related to the total endoscopic population or to the population at large.

Second, gastritis was a confused and confusing subject, virtually impossible for a clinician in training to understand from the literature available. Gastroenterologists had varying opinions on the disease, depending on which textbook they had read. The overriding view was that gastritis was a phenomenon related to aging, and that it did not cause symptoms or predispose to any significant disease entity. Perhaps because of the prevailing opinion, no one else was interested in the bacteria Warren had found. The only other person studying gastritis at our hospital was Dr. Fung,[26] but he believed it was usually caused by alcohol.

In July 1981, Warren gave me a list of 25 patients in whom large numbers of gastric spiral bacteria were present. I retrieved the 25 case notes but could not identify any characteristic clinical features in the patients. They were standard patients presenting for endoscopy, usually for epigastric pain suspected as acid peptic disease.

At the same time, I read everything available on gastritis and found no reference to the organisms. After several months, I found the beautiful photograph of a "gastric spirillum" in Ito's paper in the *Handbook of Physiology*.[25] From Ito's references I obtained a paper on cat gastric spirilla by Vial and Orrego,[28] and in their references I found the work of Palmer[11] and subsequently Freedberg and Barron,[10] Doenges,[9] Kreinitz,[3] Salomon,[2] and Bizzozero.[1] Evidently the bacteria had been discovered before!

Initially, even Warren was uncertain that the interesting spiral bacteria were important. He observed that they were beneath the mucus layer, a location that was not reached by the occasional swallowed contaminants from the mouth. In addition, the sheer numbers and homogeneity of the colonization meant that they were actually multiplying within the mucus and were obviously very well adapted to it.

We became more certain that the new bacterium was clinically relevant when we treated a patient with gastritis in September 1981. A 76-year-old Russian man with abdominal pain had been investigated extensively at the University Department of Medicine. The cause of his severe epigastric discomfort was unknown, but in view of vascular calcifications seen on x-ray, mesenteric angina was suspected. By chance, he had been included in a pilot biopsy study of 18 patients (see below). I approached Professor Laurie Beilin, his attending physician, who agreed that a course of therapy with tetracycline would be worth a try. With the patient's consent, we prescribed tetracycline syrup, 2 gm daily for 14 days. The patient's symptoms completely resolved, as did the antral gastritis when we rebiopsied him on day 14.

PILOT STUDIES—SEPTEMBER 1981

During 1981 I read extensively on the taxonomy of curved and spiral bacteria. Warren and I were both attracted to the *Campylobacter* literature because *C. jejuni* was the organism with the most similar appearance. The work of Skirrow and Benjamin[30] was already well known and campylobacters were being cultured at our hospital. I thought the bacterium might be *Campylobacter sputorum* spp. *sputorum*, which was a known mouth commensal.[31] In an informal arrangement, I obtained antral biopsies from routine endoscopy cases and carried them immediately to the microbiology department. Interest picked up a little when the microbiology lab staff could see the spiral organisms in Gram-stained squashed mucosal biopsies. In a pilot series of 18 patients the bacteria were detected on histology in about half. In only three were they seen on Gram stain, and attempts to grow them in aerobic and anaerobic environments on various media were unsuccessful. In that year, Phillips and Lee had cultured a mucosal spiral bacterium from mouse colon,[32] so we used similar microaerobic techniques. The method of obtaining a suitable gas mixture was to partially evacuate a jar and then add an anaerobic gas mixture (H_2, N_2, and CO_2). The bacterium was eventually isolated by Helen Royce and Frank Kosaris on chocolate agar in such an atmosphere (see below).

We now know that primary isolation of *C. pylori* usually takes at least three days incubation before colonies are visible on agar plates. The clinical lab at Royal Perth Hospital, used to the rapid growth seen with *Campylobacter jejuni* cultures, was discarding the plates after 48 hours if no growth was visible. In most other biological specimens, longer incubation times on nonselective media would have resulted in complete overgrowth of the plates with commensal flora. The gastric biopsy specimens, however, were very clean in this respect but were still discarded on the second or third day. About 30 unsuccessful attempts to culture the bacteria were made between August 1981 and January 1982.

BIOPSY AND CULTURE OF 100 CONSECUTIVE PATIENTS: 1982

At the suggestion of Chris Sanderson, a senior gastroenterologist at Royal Perth Hospital, I developed a protocol to formally study a series of 100 consecu-

tive patients undergoing endoscopy, aiming to (1) decide if the bacteria were associated with gastritis, (2) find the source of infection, (3) culture the bacteria, and (4) determine which diseases, if any, were associated with the infection. I asked the permission of Professor Stewart Goodwin to attempt isolation of the organism in the clinical microbiology laboratory. He allocated a technologist to the task, initially Tamara Asmutaitis and subsequently Helen Royce. The senior technologist was Frank Kosaris. Dr. John Pearman was deputized to oversee the microbiological work.

Although I was almost finished with my six-month gastroenterology term, I submitted the protocol to the Human Rights Committee of the hospital in December 1981. It was approved only after I had provided data on the risk of gastric biopsy. We reviewed the experience at Royal Perth Hospital and concluded that the chance of requiring a blood transfusion after a single antral biopsy was approximately 1:20,000 in a person without a known bleeding disorder.

The study was conducted as follows: I located all patients scheduled for elective endoscopy by studying the endoscopy appointment book. I then interviewed inpatients the night before the test and saw outpatient and emergency cases early the next morning. Patients were questioned about diet, animal contact, travel, drugs, symptoms, and dental hygiene. Only one patient refused to take part in the study.

At endoscopy, two antral biopsy specimens were taken from each patient, usually by Tom Waters or Chris Sanderson (I had by then completed my gastroenterology term and had hematology rounds each morning). One specimen was transported to the microbiology lab in nutrient broth, the other was fixed in formalin and sent to Robin Warren. In addition I took blood from all patients for later antibody studies. Data sheets were sent independently to the statistician, Norm Stenhouse (who had a duodenal ulcer himself and was quite interested in the study), and were entered by Rose Rendell. Specimen collection was supervised by the nurse on the gastroenterology unit, Dorothy "Dot" Heys, who named the organism "CB," her abbreviation for Campylo*B*acter. In retrospect, the assistance of Dot in this and future *C. pylori* studies was essential for their success. She and I made up sets of four large orange labels numbered 1–100 so that each laboratory would know they were "research" biopsies.

In January 1982 I completed my gastroenterology term and commenced a very busy hematology rotation. The study finally began in April 1982. Each week I would spend an hour or two with Robin Warren as he showed me the interesting biopsies we had received. Warren was pleased to find someone, particularly a clinician, who was as interested in the bacteria as he was. He tried to explain the terminology and classification of gastritis to me but I found it very confusing. The terms atrophic and atrophy were used interchangeably, and most of the books and papers on the subject did not provide adequate illustration of the histology discussed.

FIRST ISOLATION OF *C. PYLORI*

In April 1982, after the four-day Easter holiday (Friday through Monday in Australia), the spiral organism was cultured for the first time. The hospital was fighting an influx of methicillin-resistant *Staphylococcus aureus* at the time and,

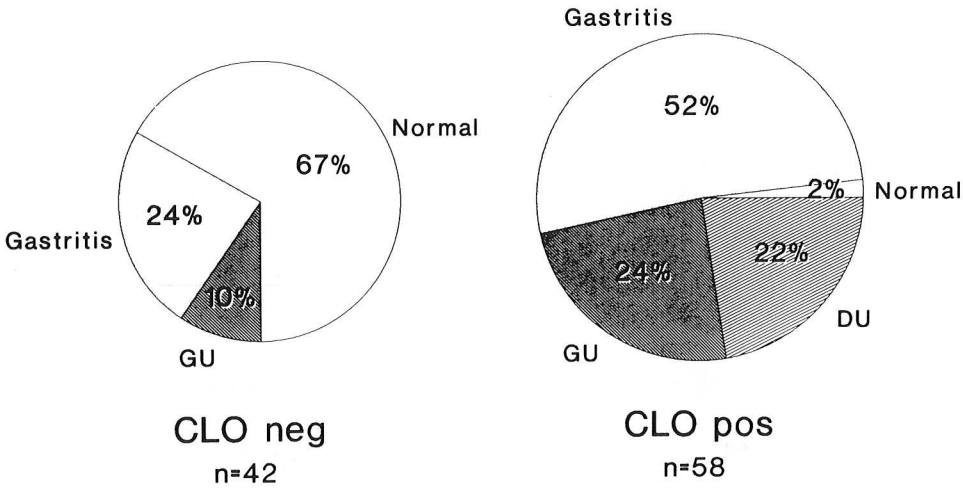

Figure 2–1 Pie chart showing distribution of spiral bacteria and gastritis in original study.

because of the extensive staff and patient surveillance being undertaken by the clinical microbiology laboratory, my project was given a lower priority. As a result, Helen Royce (or the person on call over the long weekend) had left the culture in the incubator for 5 days, long enough for *C. pylori* colonies to become visible. That first isolate is now the type strain, NCTC11637. Because of the clean specimens, overgrowth of commensals did not universally occur. Helen and Frank immediately recognized that the longer culture time was necessary and isolated the organism from a second patient that same week. The overall result of the study was that *Campylobacter*-like organisms (CLO) were seen in Gram-stained smears of 33 patients and were cultured in 11. On histology, CLO were present in specimens from 58 patients, 57 of whom had histologic gastritis. The breakdown is shown in Figure 2–1.

C. pylori had been isolated on April 8, the Tuesday after Easter. By the time Helen Royce showed me a Gram stain of the cultured organism, we had two isolates, both from biopsies in which the Gram stain had been positive. The bacteria had large bizarre forms, curved but not *Campylobacter*-like. I was not convinced that these were indeed the spiral organisms we had seen on biopsies. To check I decided to raise antibodies to them. Toward the end of April I inoculated two rabbits with one isolate each and later collected serum from them with the aim of performing immunofluorescent studies on fresh biopsy material. Cloudy suspensions of live bacteria injected on two occasions had no discernible harmful effect on the rabbits.

In parallel with the 100-patient study, sections were sent to Dr. John Armstrong, the electron microscopist, for ultrastructural examination. His department was staffed by S. H. "Willy" Wee and Dr. Bob Horne, who had been interested in the spiral bacteria since 1977 when they were seen in the gastritis study of Fung et al.[26] Mainly to satisfy his own curiosity, Wee had obtained excellent electron photomicrographs showing detailed morphology of the bacte-

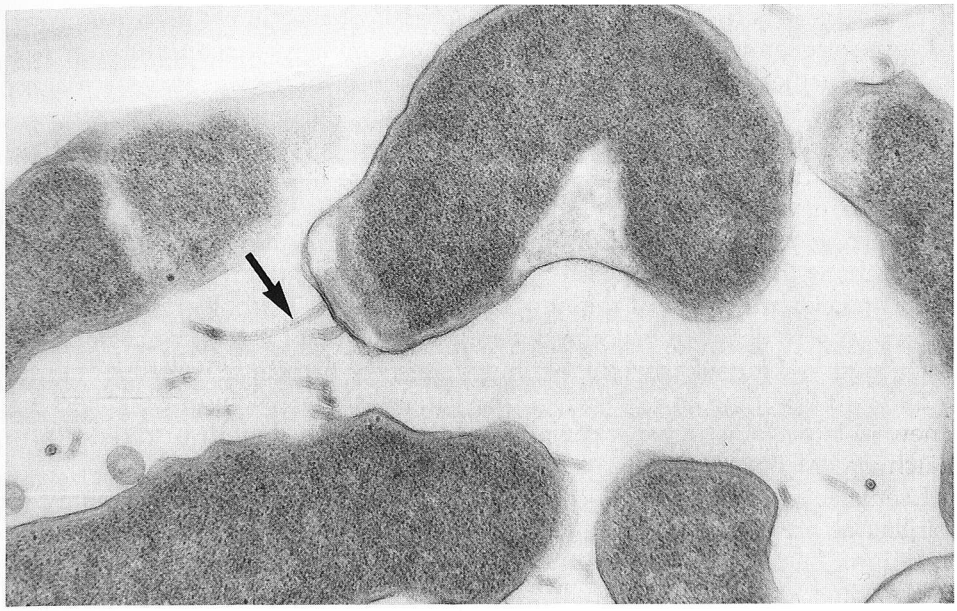

Figure 2–2 Early electron micrograph (negative stain preparation) of *C. pylori*. Note sheathed flagella (*arrow*).

ria. When the candidate organism for the gastric spiral bacterium was isolated, I delivered cultures to Armstrong, who soon identified the essential morphologic features that differentiated the gastric organism from *C. jejuni*. These were the four or five sheathed flagella, with terminal bulbs, arising from only one end of an organism without axial filaments. Figure 2–2 shows the first electron micrograph obtained from a culture of *C. pylori*.

ASSOCIATION OF *C. PYLORI* AND GASTRODUODENAL PATHOLOGY

In June 1982, the study was complete and we had biopsy data from 100 consecutive patients. My wife, Adrienne, and our four children were by then planning to move for six months to Port Hedland, a seaside mining town 1,500 miles north of Perth. This was an optional posting for final year internal medicine trainees, which paid a very good salary. In the weekend before leaving, I photocopied the endoscopy reports from the 100 patients in the study. These were the only data I took with me, as all other study data were still blinded.

Between July and August 1982 I coded the endoscopic lesions from the photocopied reports to reflect the major endoscopic findings. A list of numbers 1–100 and the associated numeric data were then mailed to Rose Rendell at Raine Foundation medical statistics unit. Concurrently, by mail, I performed an extensive literature search on campylobacters, gastritis, and the taxonomy of spiral bacteria. My contact in Perth was the Royal Perth Hospital librarian, Jean Rider-Jones. In particular, my search traced references on gastritis, microbiology of the stomach, and taxonomy of spiral bacteria. It made interesting reading, particularly the repeated observation that chronic gastritis was associated with peptic

ulcer disease. There was no mention of bacterial colonization of gastric mucosa, except as contamination from the oral flora or commensal colonization in persons with achlorhydria. My microbiology reference source was *Bergey's Manual of Determinative Microbiology*.[33] According to Bergey, the new bacterium could not be a *Campylobacter* because its flagella were sheathed. Morphologically it resembled vibrios but did not fit that genus because it was microaerophilic. Finally, the new organism was not a spirochete because it did not have axial filaments. It may have been a spirillum, a group of microaerophilic curved organisms with sheathed flagella.

For me, identification of the new bacterium posed a problem. None of the textbooks gave details concerning where to start when totally new bacteria are discovered. Most books merely told how to test if a bacterium was the same as, or different from, the known species. In a clinical laboratory this meant checking a new isolate against a panel of known pathogens and then discarding it if no match was made.

I sent Helen Royce a photocopy of the chapter in *Bergey's* on taxonomy of spirillae as an example of what I thought was necessary to characterize the bacterium. Luckily, Dr. Doug Annear had taken an interest in the new organism by this time and had successfully lyophilized the first two isolates. Helen revived these with seven other isolates and attempted characterization of the new organism. She performed a series of tests and sent me the results from nine of the eleven original isolates. I am not exactly sure how she tested the bacterium for urease, but she reported that it was urease negative. I can only guess that testing was done from a nonviable colony. We now know that *C. pylori* produces so much urease that an immediate color change occurs if a live colony is inoculated into Christensen's medium.[34]

During October 1982 I obtained the printouts from the statistician. Essentially, these were crosstabs (using the Statistical Package for the Social Sciences (SPSS)) between the clinical and endoscopic observations and the presence of gastritis and the spiral organisms. It then became clear that only people with gastritis had the gastric spiral bacteria. We therefore had an association between the bacterium and a condition that was at the time not even recognized as a disease. I was disappointed that the large amount of clinical data failed to discover a clinical syndrome associated with the new bacterium, except for the symptom of burping. Neither was there an association between the bacterium and poor dental hygiene, pets, eating chicken, drinking milk, traveling, taking cimetidine, antacids, or nonsteroidal anti-inflammatory drugs (NSAIDs). I was preparing a presentation on the study for October 1982 when, a few weeks before the abstract deadline, I noted the association between the new bacterium and peptic ulceration in a final set of crosstabs sent from the statistician. All 13 patients with duodenal ulcer had the organism.

When I observed this, I discussed it with Warren, who initially was far more cautious than I. After all, there were only 13 patients with duodenal ulcers in the study. With such a small number, the association of infection with ulcer disease could have been a fluke. However, we calculated a p value of <0.001 for the association. In addition, gastric ulcers were also associated with the bacterium, $p < 0.01$. I read further on gastritis and was impressed by the work of Magnus in the 1950s[35] and later works by Kekki et al[36] and Shrager et al.[37] It seemed too much of a coincidence that gastritis was known to be associated with peptic ulcer

disease. The modern literature had forgotten that duodenal ulcers (DU) were far more likely to be associated with gastritis than were gastric ulcers (GU). In our study, 100% of patients with DU and 80% of those with GU had the bacteria, almost identical proportions as those noted by Magnus[34] thirty years earlier.

DISSEMINATING THE FINDINGS IN AUSTRALIA

Excited, and encouraged by a number of my senior colleagues, I presented the study at the local meeting of the Royal Australian College of Physicians on October 22, 1982. The paper was well received but it was clear I was thinking very differently from the gastroenterologists. They knew about the association between gastritis and gastric ulcer, but were not aware that gastritis was present in nearly all patients with duodenal ulcers. Gastritis was so common and had so many "known" causes that to gastroenterologists our observations were perhaps equivalent to noting the presence of *E. coli* in the feces of patients with colitis.

In January 1983 I had completed my tour of duty in Port Hedland and as a result of my presentation was offered an internal medicine post at Fremantle Hospital with Ian Hislop, a gastroenterologist who had published papers on histologic gastritis in the 1960s.[38] It was a chance to learn endoscopy and further study the patients with the spiral organism. I was on call every fourth night on the general medical wards as well. Ian Hislop had busy hospital and private practices, and together we were able to biopsy many more patients and look for the curved bacteria in the early part of 1983. This enabled me to confirm the findings at Royal Perth Hospital and look more carefully at patients with peptic ulcer disease.

With my move to Fremantle Hospital, the *C. pylori* studies at Royal Perth Hospital came to a halt for about 12 months. Frankly, there was no love lost between the two institutions and I was determined to keep control of the spiral organism project. The clinical microbiologist at Fremantle Hospital was David McGechie. He had an interest in campylobacters and his laboratory was equipped with water-jacketed CO_2 incubators, which seemed to grow the spiral bacteria very well. We soon had numerous isolates growing. In retrospect, the low isolation rate in the initial study may have been because the bacteria required a very high humidity and freshly poured media for ideal growth.

During quiet nights on call, further reading lead me to the literature on duodenal ulcer healing and relapse. Although cimetidine was believed to be the "last word" in ulcer treatment, it was obvious to gastroenterologists that the acid-reducing drugs merely healed ulcers; they did not cure the disease.[39] Interestingly, however, a study by Martin et al[40] had found that when ulcers were treated with colloidal bismuth subcitrate (CBS) the relapse rate was much lower than if they had been healed with cimetidine. In fact, 30–50% of patients appeared to be cured by bismuth therapy. This suggested to me that bismuth compounds might inhibit the spiral bacteria. If spiral organisms were etiologic for the gastritis, antibacterial agents should result in mucosal healing and perhaps decrease ulcer recurrence. Conversely, drugs that did not cure ulcer disease would be expected to heal the ulcer but not the gastritis. I soon found

papers stating that cimetidine did not heal gastritis,[41] even when ulcers were healed. Another important observation, reported by MacDonald,[42] was that although gastric ulcers were associated with gastritis, about 30% of persons with gastric ulcer did not have histologic gastritis. These were usually persons taking aspirin-type medications. It seemed then that all patients with peptic ulceration had gastritis, except when the ulcer had been caused by the ingestion of corrosive medication (e.g., NSAIDs).

As I became more convinced that the spiral bacteria were an etiologic factor in peptic ulcer disease, it seemed to follow that any drug that prevented ulcer relapse must also be an antibacterial agent for spiral bacteria. To test this hypothesis, I asked one of the laboratory technologists at Fremantle Hospital, Ian Stingemore, to test liquid DeNol (CBS) (Gist Brocades, Delft, Holland) for inhibitory effect on the bacterium. Neil dipped a 5 mm disc into the liquid, dried it, then placed it into the middle of a heavily inoculated plate of *C. pylori*. Three days later I was pleased to see that a three-inch-diameter zone of inhibition was present on the plate.

In addition to obtaining gastric biopsies on all endoscopy patients, Ian Hislop and I then carried out a prospective study to see the in vivo effects of CBS on the bacterium. The study was too small to observe any significant differences in clinical events, but the histologic changes and effects on the bacteria were striking.[43]

In January 1983 I submitted a report on the spiral bacteria to the Australian Gastroenterology Society. The abstract contained the highly inflammatory statement, "They (the bacteria) may be responsible for the high relapse rate in ulcers treated with cimetidine." The abstract was reviewed by gastroenterologists and (in retrospect I can say "not surprisingly") was rejected. That same week, David McGechie received an invitation to the International Workshop on *Campylobacter* Infections and suggested that I resubmit the study to that meeting. To help, he gave me the home telephone number of Martin Skirrow. That night I called Skirrow at his home in Worcester, England and told him about the the new bacterium. We later mailed him some *C. pylori* isolates from Fremantle Hospital. One of these was from a Norwegian ex-sailor with recurrent prepyloric ulcers. That isolate is NCTC11639.

THE SECOND INTERNATIONAL WORKSHOP ON CAMPYLOBACTER INFECTIONS

In September 1983 I visited Skirrow in Worcester, one week before the second International Workshop on *Campylobacter* Infections, which was to be held in Brussels. By then, Skirrow had several of our *C. pylori* isolates growing and was quite excited about them. He and I had lunch with a dermatologist who had a reputuation as a classical scholar (and who, I believe, was instrumental in naming *Campylobacter laridis*, an organism from seagulls). Together they came up with the name *Campylobacter pyloridis*, derived from the Greek pylorus—for gatekeeper, or "one who looks both ways." We thought the name appropriate as the concept united gastric and duodenal ulcer diseases under the umbrella of gastritis and *Campylobacter* infection. Only two days before, I had received an analysis of guanine plus cytosine (G+C) content of the new organism from

Lindsay Sly in Brisbane, Australia. I made up a card with the result on it, photographed it, and Skirrow carried it to an Ektachrome laboratory in Worcester to develop the slide. The result was important because the bacterium had a G+C content of 34%, within the *Campylobacter* range, and quite incompatible with the G+C content of the morphologically similar genus, *Spirillum*. It was 1987 before the name was corrected to *Campylobacter pylori*.[44]

Skirrow arranged for me to attend an endoscopy session in Worcester with Dr. Nick Dyer, a gastroenterologist at Worcester. I obtained the necessary antral biopsies from a patient of his, an 80-year-old woman with a gastric ulcer. Skirrow's registrar, Cliodna McNulty, helped me stain the slide and we soon observed large numbers of the spiral, gram-negative organisms. She and Skirrow were able to isolate the bacteria from this patient, the first person from whom culture was attempted in the U.K. (Cliodna and Dyer proceeded with a consecutive biopsy study after that time and duplicated the results from the Royal Perth Hospital.)

A few days later I presented our report—titled "Spiral bacteria in the human stomach, a common finding in patients with gastritis and peptic ulceration"—in Brussels. In that week I met many of the current *C. pylori* investigators including Blaser (U.S.A.), Langenberg (Holland), Tytgat (Holland), Pearson (U.K.), and Butzler (Belgium). Soon after the meeting, Stewart Goodwin passed through England on sabbatical from Perth and noted the infectious enthusiasm for the new organism among English investigators. Royal Perth Hospital research into *C. pylori* accelerated after this date, and Goodwin and I collaborated in the planning of a double-blind study of antibiotic therapy for duodenal ulcer.[44]

Encouraged by Dr. Skirrow's enthusiastic response to my communications, in 1983–1984 I wrote letters to other investigators interested in gastritis. I knew that active chronic gastritis was virtually pathognomonic for infection with the gastric spiral bacteria, so I searched the gastroenterology texts looking for papers with illustrations showing classic active chronic gastritis. The two most impressive were from cases of hypochlorhydria reported by Ramsey et al[20] and Weirsinga and Tytgat.[19] I wrote to Ramsey in Dallas, Texas and Tytgat in Amsterdam and later was shown the sections from those cases. Spiral bacteria were present in most of the patients of Ramsey et al (since investigated by Walter Peterson) and in the man with Zollinger Ellison syndrome reported by Weirsinga and Tytgat.

PUBLISHING THE DATA

During 1983 Warren and I produced several drafts of a paper for submission to Lancet. By now I was convinced that the new bacterium was the primary cause of peptic ulcer disease. Our problem was to curb my enthusiasm and present the data in a form acceptable for reviewers.

In January 1984 Warren and I finally agreed on a version of the paper for submission to Lancet. In that month we were quite dismayed that a paper describing the bacteria in a series of gastric biopsies was published in the Journal of Clinical Pathology by Rollason et al.[46] However, these authors had failed to find any association between the bacteria and peptic ulcer disease, although they did describe an association with gastritis. We were annoyed that they had

not quoted our earlier letters to Lancet[47] in their references. Another major omission was that Rollason et al did not cite the work of Steer, which was puzzling, since one of Steer's papers had appeared in the same journal.[23] Steer wrote a paper on the spiral bacteria and submitted it in May 1983. He also failed to cite our 1983 letters even though his paper was amended before publication and included a reference to a 1984 Lancet letter by Phillips et al.[48] In their defense, both Rollason et al and Steer had essentially completed their manuscripts before our Lancet letters of 1983 were published.

CLOSING THE CIRCLE

In 1984, I was given a post in microbiology with David McGechie at Fremantle Hospital. With help from Peter Utley and Graham Francis, we developed a passive hemagglutination test for *C. pylori,* and we studied a group of 500 blood donors. Overall, 20% of Australian adults were infected and up to 45% of middle-aged persons had elevated antibody titers.[49] I concluded that the acute disease could not be very severe, or it would have been well-recognized in the medical texts. In the epidemic reported by Ramsey et al[21] only half of the infected cases had acute symptoms.

In early 1984, I obtained support from the Royal Perth Research Fund and attempted to infect two half-grown pigs with *C. pylori.* It was a difficult experiment. The pigs grew large and vicious, and were quite resistant to *C. pylori* infection. In desperation, I planned a self experiment in June 1984. I had already successfully eradicated the bacterium in a number of patients, so I was confident that I would come to no harm if I took the organism (a confidence I somewhat erroneously repeated to Arthur Morris). Ian Hislop performed an endoscopy on me, and on the same morning an isolate of *C. pylori* was obtained from one of his patients, an elderly man with nonulcer dyspepsia. One month later, after sensitivity tests had confirmed that the organism was susceptible to metronidazole, I omitted breakfast and premedicated myself with 800 mg Tagamet before ingesting the organisms present on a 3-day culture plate (see Chapter 5). Gastritis and hypochlorhydria developed but resolved after 14 days. Nevertheless, it had been proven that *C. pylori* could infect normal gastric mucosa and that such an infection was associated with histologic damage.

After I had performed the experiment, I was certain that the acute gastritis with hypochlorhydria described by Osler and McCrae,[5] and epidemic gastritis with hypochlorhydria described by Ramsey et al[21] and Wiersinga and Tytgat,[20] were all the same disease. In mid-1984, when the original 100-patient study was published[50] in Lancet, we were notified of the urease activity present in *C. pylori* by the letter of Langenberg.[34] Subsequent consideration of this phenomenon directed me to the extensive literature of gastric urease and a possible explanation for the hypochlorhydria associated with acute *C. pylori* infection.

In 1983, when the *C. pylori* hypothesis was developed, I was certain that it would immediately gain universal acceptance and that within two years peptic ulcer therapy would be essentially an antimicrobial regimen. Although most gastroenterologists do not yet consider *C. pylori* an important diagnosis, the exponential growth of publications on the new bacterium suggests that there is some support for its pathogenic role. The *C. pylori* story will mature, in my

opinion, when medical texts have a chapter on peptic ulcer disease within the infectious disease section.

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