

***Helicobacter pylori* and the Bacterial Theory of Ulcers**

Dr. Robin Warren

The story begins with Dr. J. Robin Warren. Dr. Warren was a pathologist at the Royal Perth Hospital (RPH) in Western Australia. A pathologist is a medical doctor who examines tissues and is responsible for the accuracy of laboratory tests. Pathologists interpret the results of these examinations and tests and provide information important for a patient's diagnosis and recovery. Part of Warren's duties as a pathologist was to examine histological sections from gastric biopsies. With the arrival in the early 1970s of the fiberoptic endoscope, it was increasingly common to find good, well-fixed sections from gastrointestinal tissue. Prior to this, it was unusual for a pathologist to see good histological sections from any part of the stomach that was not post mortem. In 1979, after examining a hematoxylin and eosin-stained section of a stomach biopsy from a man with severe gastritis, Warren noticed a thin blue line on the surface of the tissue. When he increased magnification, he thought he saw bacteria.

To get a better look at these bacteria, Warren ordered a Warthin-Starry silver stain of the histological section. Silver staining deposits silver granules on some types of bacteria, making them larger and more pronounced. The stain revealed numerous spiral shaped bacteria. This was a bit surprising since, according to medical textbooks, bacteria were not supposed to colonize the stomach. Intrigued, Warren began requesting Warthin-Starry silver stain for all the gastric biopsies he examined. He began to see a pattern. There was a definite correlation between active, chronic gastritis and the presence of these bacteria. He also observed that the number of bacteria seemed to correlate with the degree of inflammation of the stomach lining—the more severe the inflammation, the more abundant the bacteria. But this didn't make sense. Medicine taught that the stomach was sterile. It was too acidic for any bacteria to survive for very long. Bacteria in the stomach had been reported before, but dismissed as a contaminant or as secondary to the problem. Warren, however, didn't believe his spiral bacteria were simply a contaminant. Electron microscopy revealed bacteria attached to the mucosa and infiltrating between the tops of cells—an area not reached by ingestion. Also, because of the large numbers and homogeneity of colonization, he believed they were actively multiplying and living in the stomach lining.

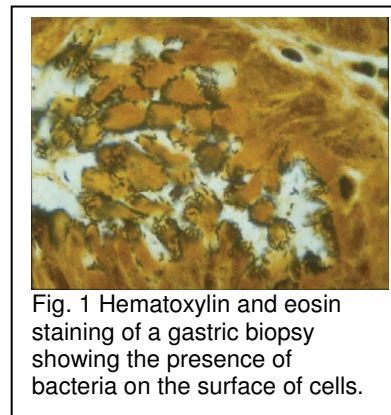


Fig. 1 Hematoxylin and eosin staining of a gastric biopsy showing the presence of bacteria on the surface of cells.

What was even more peculiar to Warren was that the bacteria were associated with gastritis. Histological gastritis is diagnosed when sections of stomach biopsies show infiltration of tissue with lymphocytic-type cells, small collections of neutrophils, micro-erosions, epithelial cell damage, and a decrease in the thickness of the mucus layer. If Warren saw this type of histological presentation in biopsies, the bacteria were usually present. When Warren discussed his findings with colleagues, they were unconvinced as to the importance of the findings and asked for more data. The stomach was a sterile environment, so Warren's bacteria were more than likely an artifact. Despite the unenthusiastic response, Warren held to his belief that these bacteria were important and tried to recruit others to his study.

Dr. Barry Marshall

In 1979, Dr. Barry Marshall moved to RPH to begin three years of internal medicine training. At RPH, doctors in the College of Physicians training program were expected to do a small research project and write a paper each year. Marshall's early attempts at research were aimed at studying heat stroke. Australian summers can get very hot, and with the popularity of short

marathons increasing, heat stroke was becoming a problem. Marshall wanted to find a way to monitor core body temperatures of runners while running. Towards this end, Marshall developed a mini-rectal thermometer attached to a piece of fishing line for easy retrieval. He and a resident spent many a day running stairs with the thermometer safely in place in hopes of raising their core body temperature to test their new monitoring system. He got a chance to use his invention during a short marathon in 1982. It was an extremely hot day and many runners collapsed as a result of heat stroke. Marshall worked on one runner, who collapsed and developed prolonged epileptic-like seizures that could be life threatening. Marshall took out his mini thermometer and recorded a core body temperature high enough to cause brain damage. With the help of bystanders, Marshall carried the man to a nearby pool to cool him off until he regained consciousness and could be transported to the hospital, where he made a full recovery.

A Partnership Begins

In 1981, Marshall was in his second year of internal medicine training and was assigned to a rotation in gastroenterology. The chief of gastroenterology suggested that Marshall work with Dr. Warren, investigating his gastric spiral bacteria. After speaking with Dr. Warren, Marshall's interest was piqued. Marshall put aside his study of heat stroke, and together he and Warren worked to isolate and study Warren's spiral-shaped bacteria.

Marshall began his research by searching the current literature. Marshall was surprised to find that Warren's spiral bacteria had already been "discovered." In 1892, the Italian pathologist Giulio Bizzozero described the presence of spiral bacteria in the stomachs of dogs. Even earlier, a Polish clinical researcher, Dr. W. Jaworski, described spiral organisms in sediments of gastric washings obtained from humans. In fact, over the years many investigators had described spiral organisms in human stomachs postmortem and in fresh surgical specimens. In the early 1970s, because of the arrival of fiber optic endoscopy, researchers began to report the presence of gram-negative bacillus in 80% of patients with gastric ulcers. However, these bacteria were thought to be pseudomonas, a common contaminant in biopsy channels of endoscopes, and therefore not significant. In addition, isolating and culturing these bacteria proved difficult, making them impossible to study. Subsequently, few took these observations seriously. Most dismissed their presence as an artifact of collecting the specimen or some type of postmortem contamination.

The Pilot Study

Marshall and Warren, however, were convinced the bacteria were important. In 1982, they initiated a pilot study of 100 consecutive patients undergoing routine endoscopy. Prior to their scheduled endoscopy, each patient was given a survey to complete. In the survey, patients were asked about their symptoms, as well as their exposure to animals, travel, dental hygiene, and diet. It was important for Warren and Marshall to know about their symptoms to determine whether there was any correlation between stated symptoms and the presence of the bacteria. But why were patients asked the other questions? Warren and Marshall needed to rule out other explanations for the presence of the bacteria. For example, at the time of the study, Kentucky Fried Chicken was the most popular fast food in Perth. Marshall felt it was important to know about patient diets, since chickens were known to possess spiral bacteria in their intestinal tract. Dental hygiene history was important, since spiral bacteria commonly inhabit dental plaque. Finally, information about the travel history of patients was important, since Warren and Marshall did not know if patients contracted the bacteria while traveling to other countries.

At the time of their endoscopy, each patient had two stomach biopsies. One was sent for sectioning and the other to the microbiology lab for culture. The study was designed to answer the following questions:

1. Are the bacteria in normal stomachs?
2. Does their presence correlate with type and severity of pathology?
3. Can the bacteria be cultured?

Isolating the Bacteria

To study a pathogen, you must first isolate it and grow it in culture. The spiral bacteria from the stomach biopsies proved difficult to isolate. Warren and Marshall believed the bacteria to be a type of *Campylobacter*. Warren's bacteria were similar in appearance to *Campylobacter jejuni*, a type of bacteria known to cause intestinal problems. At RPH, *Campylobacter jejuni* was routinely being isolated from stool samples sent to the microbiology lab. *Campylobacters*, however, are notoriously difficult to culture. They require a microaerobic technique, which requires the growth chamber to be evacuated of air and an atmosphere of less than 5% oxygen pumped in. By April 1982, Warren and Marshall had attempted to culture samples from about 30 patients without success.

Then Easter arrived. The hospital at the time was fighting an outbreak of *Staphylococcus aureus*, and the staff by mistake left the latest culture attempt in the incubator over the holidays. The normal procedure was to leave cultures to incubate for two days. If after two days no growth was evident, the cultures were discarded. But with the holiday and the *Staphylococcus aureus* outbreak, cultures were left in the incubator for 5 days. When the staff came back to discard the cultures, they were surprised to find growth. Apparently, Warren's spiral bacteria were slow growers, and it took 5 days for the colonies to emerge. Lab technicians, following standard protocol, had been throwing out viable cultures for the last 6 months!

Warren and Marshall were ecstatic. After months of effort, they had finally isolated and cultured their bacteria. Marshall had specimens sent to an electron microscopist. The results indicated it was similar to *Campylobacter jejuni*, but there were some key differences. The main one was the number and type of flagella. Warren and Marshall's bacteria had 4 sheathed flagella at one end. *Campylobacter jejuni* has only one unsheathed flagellum at one or both ends. The new isolate was named *Campylobacter pyloridis* because of its similarity to *Campylobacter jejuni*. The name was later changed to *Campylobacter pylori*. However, after additional studies, *Campylobacter pylori* was found not to be a *Campylobacter* at all and renamed *Helicobacter pylori*.

The Data

The analysis of the pilot study continued. The data was sent to a statistician to look for any relationship between the clinical and endoscopic results and the presence of gastritis and *Helicobacter pylori*. The data, as presented in the original paper published in *The Lancet*, can be seen in the Table (Fig. 3).

Based on their data and the statistical analysis, Warren and Marshall concluded the following:

- a. There were no well-defined clinical symptoms associated with the presence of the bacteria except for burping and abdominal pain.
- b. There was no evidence that other sources of infections such as animal contact, diet, and poor dental hygiene were the source of the infection.
- c. Gastritis, which was histologically diagnosed, was closely associated with the presence of the bacteria.
- d. Bacteria were present almost exclusively in patients with chronic gastritis and were also common in those with peptic ulceration of the stomach or duodenum.

In fact, 26 out of 30 patients with ulcers had the bacteria. Those four patients with ulcers but negative for the bacteria had taken non-steroidal, anti-inflammatory drugs known to induce ulcers. Warren and Marshall now felt they had sufficient evidence linking *Helicobacter pylori* with gastritis and peptic ulcer disease. The next step was to present their findings.

Fig. 3: Data Tables Published in The Lancet by Warren and Marshall in 1984

Endoscopic Appearance*	Total	With Bacteria	p
Gastric ulcer	22	18 (77%)	0.0086
Duodenal ulcer	13	13 (100%)	0.00044
All ulcers	31	27 (87%)	0.00005
Oesophagus abnormal	34	14 (41%)	0.996
Gastritis [†]	42	23 (55%)	0.78
Duodenitis [†]	17	9 (53%)	0.77
Bile in stomach	12	7 (58%)	0.62
Normal	16	8 (50%)	0.84
Total	100	58 (58%)	

*More than one description applies to several patients.
[†]Refers to endoscopic appearance, not histological inflammation.

Gastritis	Bacterial Grade				total
	Nil	1+	2+	3+	
Normal*	29	2	0	0	31
Chronic [†]	12	9	7	1	29
Active	2	5	15	18	40
Total	43	16	22	19	100

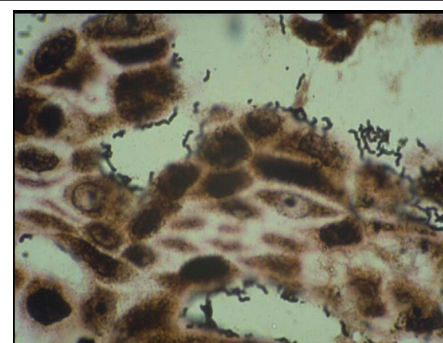
*Gastritis grades 0 and 1 are normal.
[†]1 case showed bacteria on gram stained smear.

Presenting Their Findings

Marshall and Warren presented their findings to the College of Physicians at RPH. Their work was not well received. Most were skeptical of their claims. Of the many objections, the main one was the link between gastritis and duodenal ulcers. Prevailing wisdom said that gastritis was associated with gastric ulcers, not duodenal ulcers. Marshall did further research and found a study done at the Mayo Clinic in the 1950s. In this study, stomachs from either motor vehicle fatalities or partial gastrectomy patients were examined for the presence of gastritis and ulcers. Researchers found a surprisingly high number of specimens that showed evidence of gastritis. Of those with duodenal ulcers, 100% (N = 250) also had gastritis. Here was evidence that supported Warren and Marshall's hypothesis. Why had the ulcer research community skipped over the relationship between gastritis and duodenal ulcer? Marshall speculated it was because it didn't fit with current beliefs and was therefore ignored.

Marshall continued reviewing the literature for any information pertaining to their research. He read that while acid reducing drugs relieved the symptoms of the ulcers, they did not cure them. The relapse rate was high. Cimetidine, a common acid-reducing agent, helped to heal the ulceration, but did not cure the disease. Marshall was further intrigued when he read that colloidal bismuth subcitrate (CBS; similar to the over-the-counter drug PeptoBismol) greatly reduced the relapse rate in those with gastric ulcers. Marshall hypothesized that if a drug lowered

the relapse rate for gastric ulcers and if bacteria can cause gastric ulcers, then CBS should have anti-microbial properties. To test this, Marshall soaked a filter paper disc in CBS and then placed it in the middle of a Petri dish inoculated with *Helicobacter pylori*. A few days later, much to his delight, there was a clear zone of inhibition around the paper disc while



Silver Stain of *H. pylori* on gastric mucus-secreting epithelial cells of Dr. Marshall's stomach biopsy taken 8 days after he drank a culture of *H. pylori* (x1000)

cimetidine had no effect. This provided further evidence supporting the bacterial theory of ulcers. Warren and Marshall needed more evidence. They needed to show a causal relationship between contracting *Helicobacter pylori* and developing gastritis. Marshall decided to use pigs as his model system. Pigs suffer from peptic ulcer disease just like humans, and they are large enough to be endoscoped. Marshall tried to infect two pigs with *Helicobacter pylori*, but neither developed gastritis nor gastric ulcers. Also, as the pigs grew larger, it became dangerous to work with them. Marshall needed to show that *Helicobacter pylori* caused gastritis. So, in desperation, Marshall submitted to a self-experiment. He knew that *Helicobacter pylori* were sensitive to several antibiotics, and he had successfully treated patients with gastritis using antibiotics. The culture he was eventually to drink was isolated from a man diagnosed with active gastritis. The organism was found to be sensitive to the antibiotic metronidazole. The man was given a two-week treatment of bismuth and metronidazole and cured of the disease. Therefore, Marshall felt confident that if he became infected with the same culture, he would be able to cure himself.

First, Marshall submitted himself to an endoscopic examination. The results, as expected, indicated no evidence of inflammation. A month later, after pre-medicating himself with the anti-acid drug cimetidine, Marshall drank 30 mLs of peptone broth containing *Helicobacter pylori* from a 3-day culture isolated from the patient mentioned above.

Seven days after drinking the bacterial cocktail, Marshall became sick and began to vomit. After ten days, still quite ill, he underwent a second endoscopic biopsy of his stomach. The results, much to his satisfaction, revealed gastritis and the presence of *Helicobacter pylori*. A few days later, at the insistence of his wife, Marshall began antibiotic treatments. Soon after, his symptoms disappeared. Marshall underwent an additional endoscopy, but none of the histological sections showed the presence of *Helicobacter pylori*; it had totally disappeared. But Warren and Marshall now had evidence linking *Helicobacter pylori* infection of a normal stomach to gastric inflammation.

Presenting their Results

Warren and Marshall had a difficult time getting their work published. They submitted an abstract of their research for presentation at a meeting of the Gastroenterological Society of Australia, only to have it rejected. The reason cited was the large number of abstracts submitted for the meeting. In the rejection letter, the selection committee stated that of the 67 abstracts submitted they were able to accept 56. Apparently, the committee felt Warren and Marshall's paper to be in the bottom 10%, not important enough to be presented at the meeting. They rewrote their abstract and submitted it to the International *Campylobacter* Workshop in Brussels with more success. After the pilot study was completed, Warren and Marshall submitted their work to the medical journal, *The Lancet*. Editors were initially reluctant to publish their work because they could find no reviewers who believed their results. Dr. Martin Skirrow, Chair of the Brussels *Campylobacter* conference, was contacted about their problem. He had their work repeated in his laboratory and found similar results. Dr. Skirrow contacted *The Lancet* confirming Warren and Marshall's findings and, in June 1984, Warren and Marshall succeeded in publishing their paper titled "Unidentified Curved Bacilli in the Stomach of Patients with Gastritis and Peptic Ulceration."

In Conclusion

By 1994, after many more years of research by Warren, Marshall, and others, the medical community began to accept their bacterial theory of ulcers. The following quotation by the noted gastroenterologist, Dr. Ransohoff, suggests how difficult it was for the medical community to give up their beliefs:

The long-held hypothesis that duodenal ulcer disease is caused primarily by acid has, after a decade of siege by the Helicobacter pylori hypothesis, finally collapsed. That the acid hypothesis could even be challenged, much less toppled, appeared as unthinkable 10 years ago as the fall of Communism in the former USSR. Within the last few years, strong evidence has accumulated, however, about Helicobacter pylori's importance, persuading even this previously skeptical writer.

The impact of Warren and Marshall's bacterial theory of ulcers is profound. The medical community thought peptic ulcer disease was caused by excess stomach acid. Drug companies spent millions researching and marketing drugs like Tagamet to treat the disease. Patients, in extreme cases, even underwent surgery to cut nerves involved in stimulating acid production. While these treatments did alleviate the symptoms, they did not cure the disease. Relapse rates were always high, prolonging patient suffering. By altering the way the medical community looked at peptic ulcer disease, patients are being spared unnecessary pain and anguish. Because of Warren and Marshall's ideas, the medical community has changed the way they look at ulcers. Now the standard treatment regime for peptic ulcer disease is long-term antibiotics. This has resulted in a significantly reduced relapse rate for the disease, reduced medical costs, and, more importantly, reduced patient suffering.



Dr. Robin Warren (left) and Dr. Barry Marshall celebrating winning the 2005 Nobel Prize for Medicine.

Questions

1. Do you think it ethical and appropriate for Marshall to have used himself as a test subject and swallowed a sample of *Helicobacter pylori*? What precautions did he take? Would you do it? Why or why not? **Surnames from Starting with A - B**
- 2) What causes ulcers? **Surnames from Starting with C - D**
3. How did the colloidal bismuth subcitrate (CBS) experiment provide evidence supporting Warren and Marshall's hypothesis? **Surnames from Starting with E - G**
4. Answer the following questions based on the data presented in the Table:
 - a. In Table II, of those patients with ulcers, how many were positive for *H. pylori*? Of those patients with normal endoscopic results, how many were positive for the bacteria?
 - b. Based on this data, Warren and Marshall hypothesized that there was a causal relationship between ulcers and bacterial infection. But there were 4 patients with ulcers that were negative for the bacteria. Why is this not significant?
 - c. If there is a causal relationship between the presence of *H. pylori* and ulcers, how might you explain that 50% of the patients with a normal endoscopic examination were infected with the bacteria?
 - d. In your own words, explain the results presented in Table III. What do you conclude from this data?
5. Which is a good way to test the "excess" acid hypothesis? **Surnames from Starting with H - I**
6. Robert Koch was a German physician who identified the bacteria causing anthrax and tuberculosis. His methods established four criteria that must be met for a specific pathogen to be considered the cause of a disease. These four criteria are listed below. For each one, discuss whether Warren and Marshall fulfilled them and, if so, how.
 - I. The pathogen should be found in the bodies of animals having the disease.
 - II. The suspected pathogen should be obtained from the diseased animal and grown outside the body.
 - III. The inoculation of that pathogen, grown in pure cultures, should produce the disease in an experimental animal.
 - IV. The same pathogen should be isolated from the experimental animal after the disease develops.**Surnames from Starting with J - K**
7. What role did chance, assumptions, and curiosity play in Warren and Marshall's research on *Helicobacter pylori*? **Surnames from Starting with L - M**
8. Dr. Warren's colleagues did not believe there were bacteria in the stomach. Why do you think other pathologists did not believe bacteria were in the stomach of biopsies? What alternative explanations can you provide? **Surnames from Starting with N - R**

9. If you were working with Drs. Warren and Marshall to design a study to determine whether the bacteria caused ulcers, how would you do it? **Surnames from Starting with S - T**
10. How does this case illustrate the role of technology in scientific progress? **Surnames from Starting with V - Z**
11. Why is this discovery significant? Do you think it is worthy of a Nobel Prize?
11. Albert Gyorgyi, 1937 Nobel Laureate in Physiology and Medicine, once said in his Nobel award speech:
“Discovery consists of seeing what everybody has seen and thinking what nobody has thought.” Describe how this statement applies to Warren and Marshall’s pioneering work on peptic ulcer disease.