

The Bacteria behind Ulcers

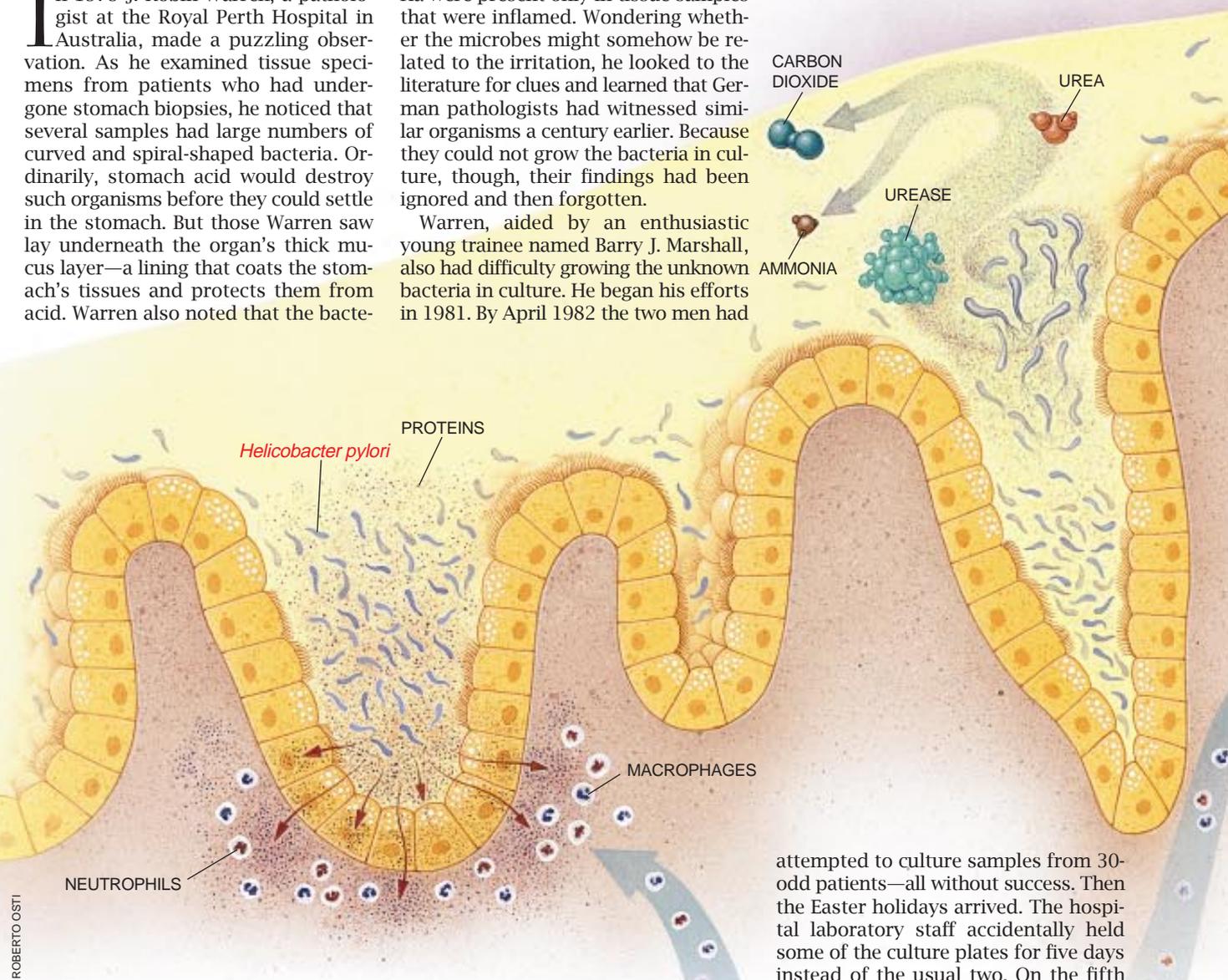
One half to one third of the world's population harbors Helicobacter pylori, "slow" bacteria that infect the stomach and can cause ulcers and cancer there

by Martin J. Blaser

In 1979 J. Robin Warren, a pathologist at the Royal Perth Hospital in Australia, made a puzzling observation. As he examined tissue specimens from patients who had undergone stomach biopsies, he noticed that several samples had large numbers of curved and spiral-shaped bacteria. Ordinarily, stomach acid would destroy such organisms before they could settle in the stomach. But those Warren saw lay underneath the organ's thick mucus layer—a lining that coats the stomach's tissues and protects them from acid. Warren also noted that the bacte-

ria were present only in tissue samples that were inflamed. Wondering whether the microbes might somehow be related to the irritation, he looked to the literature for clues and learned that German pathologists had witnessed similar organisms a century earlier. Because they could not grow the bacteria in culture, though, their findings had been ignored and then forgotten.

Warren, aided by an enthusiastic young trainee named Barry J. Marshall, also had difficulty growing the unknown bacteria in culture. He began his efforts in 1981. By April 1982 the two men had



ULCER-CAUSING BACTERIA (*Helicobacter pylori*) live in the mucus layer (pale yellow) lining the stomach. There they are partially protected from the stomach's acid (pink). The organisms secrete proteins that interact with the stomach's epithelial cells and attract macrophages and neutrophils, cells that cause inflammation (left). The bacteria further produce urease, an enzyme that helps to break down urea into ammonia and carbon dioxide; ammonia can neutralize stomach acid (center). *H. pylori* also secrete toxins that contribute to the formation of stomach ulcers (right). The microbes typically collect in the regions shown in the diagram at the far right.

attempted to culture samples from 30-odd patients—all without success. Then the Easter holidays arrived. The hospital laboratory staff accidentally held some of the culture plates for five days instead of the usual two. On the fifth day, colonies emerged. The workers christened them *Campylobacter pyloridis* because they resembled pathogenic bacteria of the *Campylobacter* genus found in the intestinal tract. Early in 1983 Warren and Marshall published their first report, and within months scientists around the world had isolated

the bacteria. They found that it did not, in fact, fit into the *Campylobacter* genus, and so a new genus, *Helicobacter*, was created. These researchers also confirmed Warren's initial finding, namely that *Helicobacter pylori* infection is strongly associated with persistent stomach inflammation, termed chronic superficial gastritis.

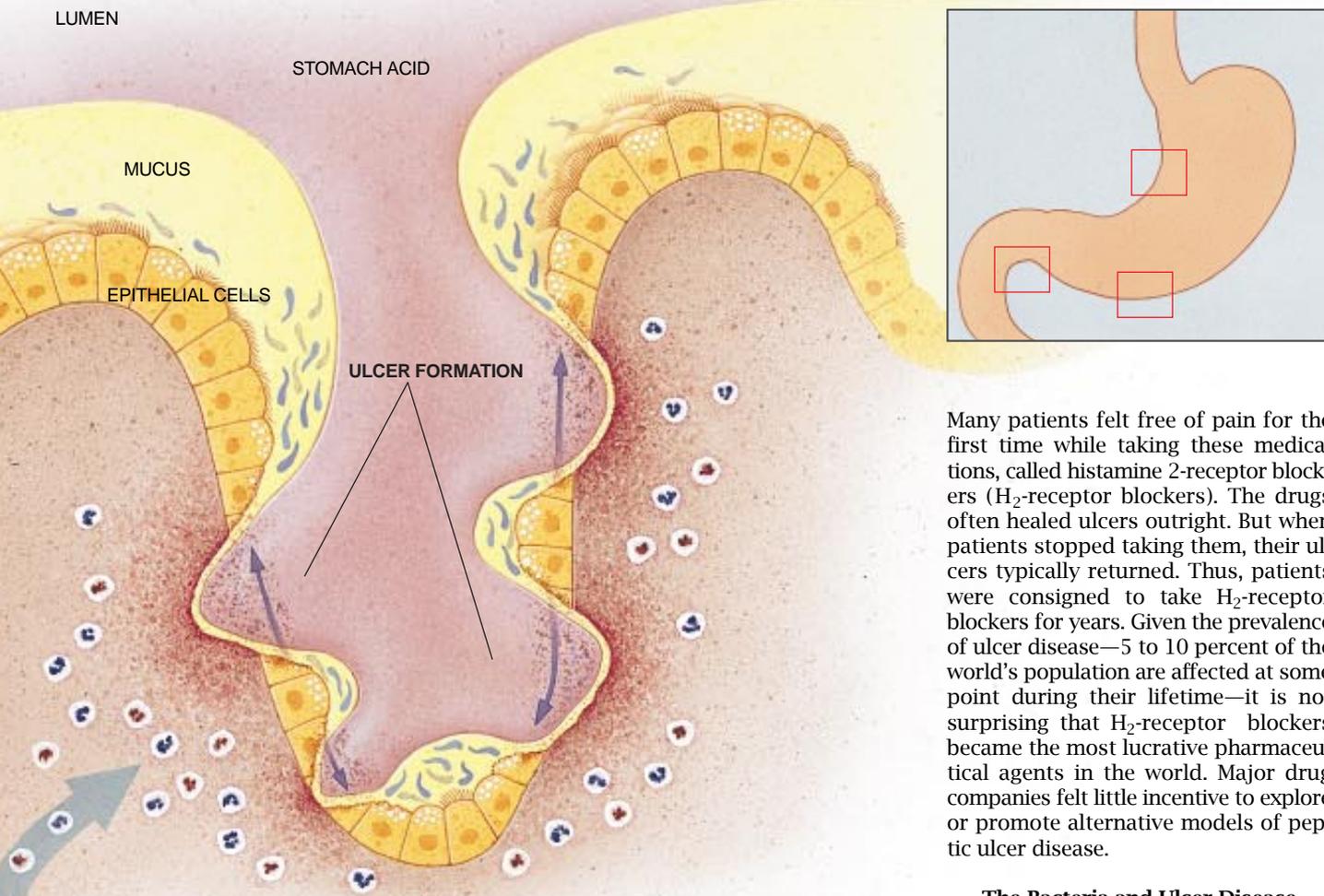
The link led to a new question: Did

acquire chronic superficial gastritis. Left untreated, both the infection and the inflammation last for decades, even a lifetime. Moreover, this condition can lead to ulcers in the stomach and in the duodenum, the stretch of small intestine leading away from the stomach. *H. pylori* may be responsible for several forms of stomach cancer as well.

More than 40 years ago doctors rec-

essary for ulcers to form, it is not sufficient to explain their occurrence—most patients with ulcers have normal amounts of stomach acid, and some people who have high acid levels never acquire ulcers.

Nevertheless, the stress-acid theory of ulcers gained further credibility in the 1970s, when safe and effective agents to reduce gastric acid were introduced.



the inflamed tissue somehow invite *H. pylori* to colonize there, or did the organisms actually cause the inflammation? Research proved the second hypothesis correct. In one of the studies, two male volunteers—Marshall included—actually ingested the organisms [see box on next page]. Both had healthy stomachs to start and subsequently developed gastritis. Similarly, when animals ingested *H. pylori*, gastritis ensued. In other investigations, antibiotics suppressed the infection and alleviated the irritation. If the organisms were eradicated, the inflammation went away, but if the infection recurred, so did the gastritis. We now know that virtually all people infected by *H. pylori*

recognized that most people with peptic ulcer disease also had chronic superficial gastritis. For a variety of reasons, though, when the link between *H. pylori* infection and gastritis was established, the medical profession did not guess that the bacteria might prompt peptic ulcer disease as well. Generations of medical students had learned instead that stress made the stomach produce more acid, which in turn brought on ulcers. The theory stemmed from work carried out by the German scientist K. Schwartz. In 1910, after noting that duodenal ulcers arose only in those individuals who had acid in their stomachs, he coined the phrase “No acid, no ulcer.” Although gastric acidity is nec-

Many patients felt free of pain for the first time while taking these medications, called histamine 2-receptor blockers (H_2 -receptor blockers). The drugs often healed ulcers outright. But when patients stopped taking them, their ulcers typically returned. Thus, patients were consigned to take H_2 -receptor blockers for years. Given the prevalence of ulcer disease—5 to 10 percent of the world's population are affected at some point during their lifetime—it is not surprising that H_2 -receptor blockers became the most lucrative pharmaceutical agents in the world. Major drug companies felt little incentive to explore or promote alternative models of peptic ulcer disease.

The Bacteria and Ulcer Disease

In fact, ulcers can result from medications called nonsteroidal anti-inflammatory agents, which include aspirin and are often used to treat chronic arthritis. But all the evidence now indicates that *H. pylori* cause almost all cases of ulcer disease that are not medication related. Indeed, nearly all patients having such ulcers are infected by *H. pylori*, versus some 30 percent of age-matched control subjects in the U.S., for example. Nearly all individuals with ulcers in the duodenum have *H. pylori* present there. Studies show that *H. pylori* infection and chronic gastritis increase from three to 12 times the risk of a peptic ulcer developing within 10 to 20 years of infection with the bacte-



Don't Try This at Home

Barry J. Marshall (*left*) of the Royal Perth Hospital in Australia made headlines after he announced in 1985 that he had ingested *Helicobacter pylori*. Marshall hoped to demonstrate that the bacteria could cause peptic ulcer disease. Marshall did in fact develop a severe case of gastritis, but the painful inflammation vanished without treatment.

Two years later Arthur J. Morris and Gordon I. Nicholson of the University of Auckland in New Zealand reported the case of another volunteer who wasn't so lucky. This man, a healthy 29-year-old, showed signs of infection for only 10 days, but his condition lasted much longer. On the 67th day of infection, the volunteer started treatment with bismuth subsalicylate (Pepto-Bismol). Five weeks later a biopsy indicated that the medication had worked. But a second biopsy taken nine months after the first showed that both the infection and the gastritis had recurred. Only when the subject received two different antibiotics as well as bismuth subsalicylate was his infection finally cured three years later. —M.J.B.

ria. Most important, antimicrobial medications can cure *H. pylori* infection and gastritis, thus markedly lowering the chances that a patient's ulcers will return. But few people can overcome *H. pylori* infection without specific antibiotic treatment.

When someone is exposed to *H. pylori*, his or her immune system reacts by making antibodies, molecules that can bind to and incapacitate some of the invaders. These antibodies cannot eliminate the microbes, but a blood test readily reveals the presence of antibodies, and so it is simple to detect infection. Surveys consistently show that one third to one half of the world's population carry *H. pylori*. In the U.S. and western Europe, children rarely become infected, but more than half of all 60-year-olds have the bacteria. In contrast,

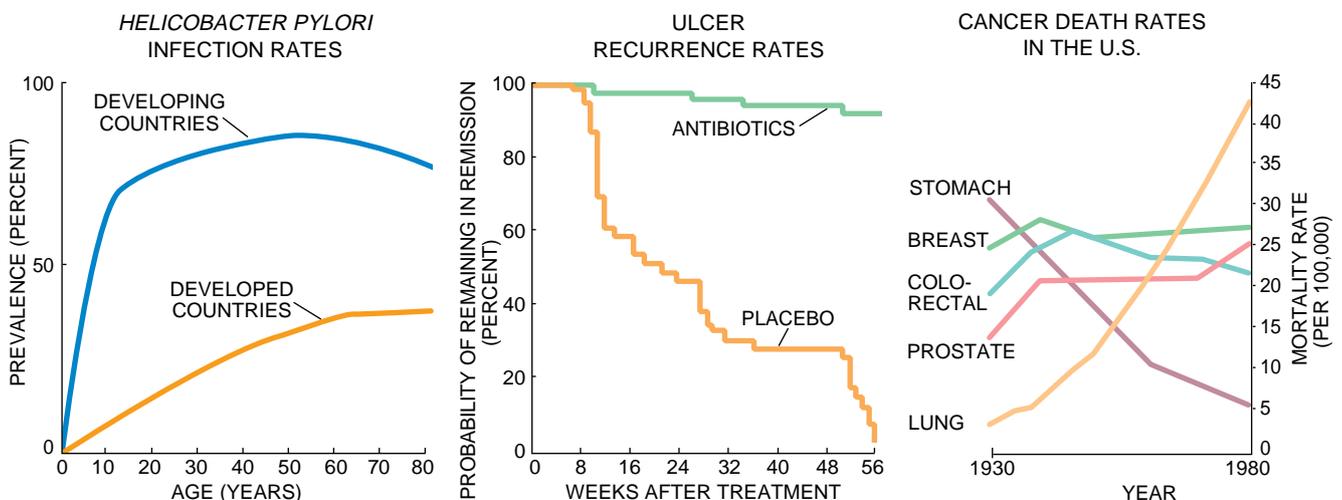
60 to 70 percent of the children in developing countries show positive test results by age 10, and the infection rate remains high for adults. *H. pylori* infection is also common among institutionalized children.

Although it is as yet unclear how the organisms pass from one person to another, poor sanitation and crowding clearly facilitate the process. As living conditions have improved in many parts of the world during the past century, the rate of *H. pylori* infection has decreased, and the average age at which the infection is acquired has risen. Gastric cancer has also become progressively less common during the past 80 years. At the start of the 20th century, it was the leading cause of death from cancer in the U.S. and many other developed countries. Now it is far down on

the list. The causes for its decline are not well understood, but we have reason to believe that the drop in *H. pylori* infection rates deserves some credit.

A Connection to Cancer

In the 1970s Pelayo Correa, now at Louisiana State University Medical Center, proposed that gastric cancer resulted from a series of changes in the stomach taking place over a long period. In Correa's model, a normal stomach would initially succumb to chronic superficial gastritis for unknown reasons. We now know that *H. pylori* are to blame. In the second step—lasting for perhaps several decades—this gastritis would cause more serious harm in the form of a lesion, called atrophic gastritis. This lesion might then lead to fur-



RATES OF INFECTION with *H. pylori* vary throughout the world. In developed countries, the infection is rare among children, but its prevalence rises with age. In developing countries, far more people are infected in all age groups (*left*). Supporting the fact that such infections cause ulcer disease, Enno Hentschel and his colleagues at Hanusch Hospital

in Vienna found that antimicrobial therapy dramatically decreased the chance that a duodenal ulcer would recur (*center*). As infection rates have declined during the past century in the U.S., so, too, have the number of deaths from stomach cancer (*right*)—suggesting that *H. pylori* infection can, under some circumstances, cause that disease as well.

ther changes, among them intestinal metaplasia and dysplasia, conditions that typically precede cancer. The big mystery since finding *H. pylori* has been: Could the bacteria account for the second transition—from superficial gastritis to atrophic gastritis and possibly cancer—in Correa's model?

The first real evidence linking *H. pylori* and gastric cancer came in 1991 from three separate studies. All three had similar designs and reached the same conclusions, but I will outline the one in which I participated, working with Abraham Nomura of Kuakini Medical Center in Honolulu. I must first give some background. In 1942, a year after the bombing of Pearl Harbor, the selective service system registered young Japanese-American men in Hawaii for military service. In the mid-1960s medical investigators in Hawaii examined a large group of these men—those born between 1900 and 1919—to gain information on the epidemiology of heart disease, cancer and other ailments. By the late 1960s they had assembled a cohort of about 8,000 men, administered questionnaires and obtained and frozen blood samples. They then tracked and monitored these men for particular diseases they might develop.

For many reasons, by the time we began our study we had sufficient information on only 5,924 men from this original group. Among them, however, 137 men, or more than 2 percent, had acquired gastric cancer between 1968 and 1989. We then focused on 109 of these patients, each of whom was matched with a healthy member of the cohort. Next, we examined the blood samples frozen in the 1960s for antibodies to *H. pylori*. One strength of this study was that the samples had been taken from these men, on average, 13 years before they were diagnosed with cancer. With the results in hand, we asked the critical question: Was evidence of preexisting *H. pylori* infection associated with gastric cancer? The answer was a strong yes. Those men who had a prior infection had been six times more likely to acquire cancer during the 21-year follow-up period than had men showing no signs of infection. If we confined our analysis to cancers affecting the lower part of the stomach—an area where *H. pylori* often collect—the risk became 12 times as great.

The other two studies, led by Julie Parsonnet of Stanford University and by David Forman of the Imperial Cancer Research Fund in London, produced like findings but revealed slightly lower risks. Over the past five years, further epidemiological and pathological investigations have confirmed the associa-

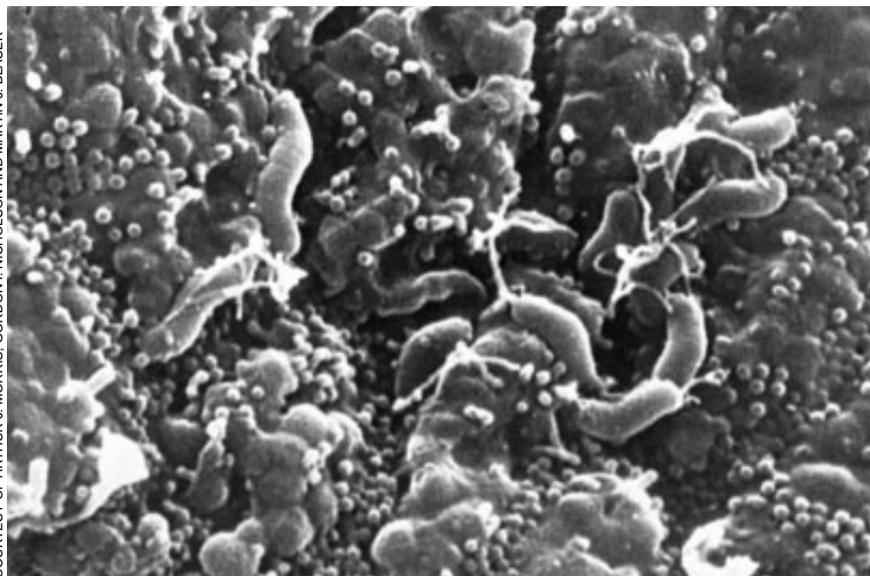
tion of *H. pylori* infection and gastric cancer. In June 1994 the International Agency for Research in Cancer, an arm of the World Health Organization, declared that *H. pylori* is a class-I carcinogen—the most dangerous rank given to cancer-causing agents. An uncommon cancer of the stomach, called gastric lymphoma, also appears to be largely caused by *H. pylori*. Recent evidence suggests that antimicrobial treatment to cure *H. pylori* infection may bring about regression in a subset of tumors of this kind, which is an exciting development in both clinical medicine and cancer biology.

How Persistence Takes Place

Certainly most bacteria cannot survive in an acidic environment, but *H. pylori* are not the only exception. Since that bacteria's discovery, scientists have isolated 11 other organisms from

dioxide. Fostering the production of ammonia may be one way helicobacters neutralize the acid in their local environment, further securing their survival.

An interesting puzzle involves what *H. pylori* eat. There are two obvious guesses: the mucus in which it lives and the food its human host ingests. But Denise Kirschner of Texas A&M University and I constructed a mathematical model showing that *H. pylori* would not be able to persist for years relying on those nutrient sources. In our model, the mathematics of persistence in the stomach requires some regulated interaction between the host cells and the bacteria. Inflammation provides one such interaction, and so I have proposed that *H. pylori* might trigger inflammation for the purpose of acquiring nutrients. An apparent paradox in *H. pylori* biology is that although the organisms do not invade the gastric tissue, they can cause irritation there. Rather, as we



COURTESY OF ARTHUR J. MORRIS, GORDON I. NICHOLSON AND MARTIN J. BLASER

CURVED ORGANISMS, magnified 8,000 times, are *H. pylori* in the stomach of the second human volunteer to ingest the bacteria. The man had consumed the microbes 463 days earlier and developed chronic superficial gastritis as a result.

the stomachs of other primates, dogs, cats, rodents, ferrets and even cheetahs. These bacteria, for now considered to be members of the *Helicobacter* family, seem to have a common ancestor. All are spiral-shaped and highly motile (they swim well)—properties enabling them to resist the muscle contractions that regularly empty the stomach. They grow best at oxygen levels of 5 percent, matching the level found in the stomach's mucus layer (ambient air is 21 percent oxygen). In addition, these microbes all manufacture large amounts of an enzyme called urease, which cleaves urea into ammonia and carbon

and others have found, the microbes release chemicals that the stomach tissue absorbs. These compounds attract phagocytic cells, such as leukocytes and macrophages, that induce gastritis.

The host is not entirely passive while *H. pylori* bombard it with noxious substances. Humans mount an immune response, primarily by making antibodies to the microbe. This response apparently does not function well, though, because the infection and the antibodies almost inevitably coexist for decades. In essence, faced with a pathogen that cannot be easily destroyed, humans had two evolutionary options: we could

have evolved to fight *H. pylori* infection to its death, possibly involving the abrogation of normal gastric function, or we could have become tolerant and tried to ignore the organisms. I believe the choice was made long ago in favor of tolerance. The response to other persistent pathogens—such as the microbes responsible for malaria and leprosy—may follow the same paradigm, in which it is adaptive for the host to dampen its immune reaction.

Fortunately, it is not in *H. pylori*'s best interest to take advantage of this passivity, growing to overwhelming numbers and ultimately killing its host. First, doing so would limit the infection's opportunity to spread. Second, even in a steady state, *H. pylori* reaches vast numbers (from 10^7 to 10^{10} cells) in the stomach. And third, further growth might exhaust the mechanisms keeping the immune system in check, leading to severe inflammation, atrophic gastritis and, eventually, a loss of gastric acidity. When low acidity occurs, bacteria from the intestines, such as *Escherichia coli*, are free to move upstream and colonize the stomach. Although *H. pylori* can easily live longer than *E. coli* in an acid environment, *E. coli* crowds *H. pylori* out of more neutral surroundings. So to avoid any competition with intestinal bacteria, *H. pylori* must not cause too much inflammation, thereby upsetting the acid levels in the stomach.

Are *H. pylori* symbionts that have only recently evolved into disease-causing organisms? Or are they pathogens on the long and as yet incomplete road toward symbiosis? We do not yet know, but we can learn from the biology of *Mycobacterium tuberculosis*, the agent responsible for tuberculosis. It, too, infects about one third of the world's population. But as in *H. pylori* infection, only 10 percent of all infected people become sick at some point in their life; the other 90 percent experience no symptoms whatsoever. The possible explanations fall into several main categories. Differences among microbial strains or among hosts could explain why some infected people acquire certain diseases and others do not. Environmental cofactors, such as whether someone eats well or smokes, could influence the course of infection. And the age at which someone acquires an infection might alter the risks. Each of these categories affects the outcome of *H. pylori* infection, but I will describe in the next section the microbial differences.

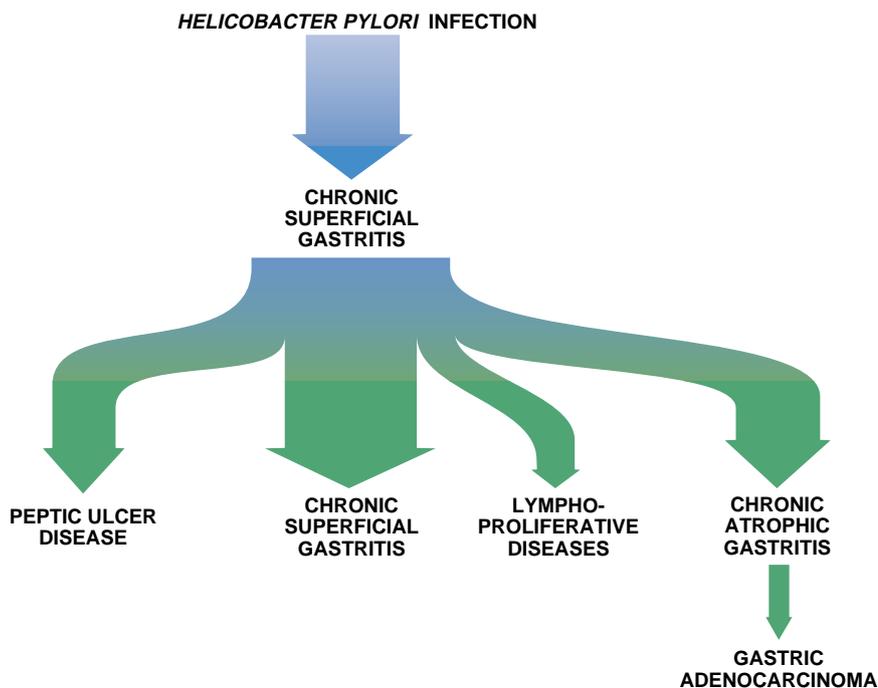
Not All Bacteria Are Created Equal

Given its abundance throughout the world, it is not surprising that *H. pylori* are highly diverse at the genetic level. The sundry strains share many structural, biochemical and physiological characteristics, but they are not all

equally virulent. Differences among them are associated with variations in two genes. One encodes a large protein that 60 percent of all strains produce. Our group at Vanderbilt University, comprising Murali Tummuru, Timothy L. Cover and myself, and a group at the company Biocine in Italy, led by Antonello Covacci and Rino Rappuoli, identified and cloned the gene nearly simultaneously in 1993 and by agreement called it *cagA*. Among patients suffering from chronic superficial gastritis alone, about 50 to 60 percent are infected by *H. pylori* strains having the *cagA* gene. In contrast, nearly all individuals with duodenal ulcers bear *cagA* strains. Recently we reexamined the results of the Hawaiian study and found that infection by a *cagA* strain was associated with a doubled risk of gastric cancer. Research done by Jean E. Crabtree of Leeds University in England and by the Vanderbilt group has shown that persons infected by *cagA* strains experience more severe inflammation and tissue injury than do those infected by strains lacking the *cagA* gene.

The other *H. pylori* gene that seems to influence disease encodes for a toxin. In 1988 Robert D. Leunk, working for Procter & Gamble—the makers of bismuth subsalicylate (Pepto-Bismol)—reported that a broth containing *H. pylori* could induce the formation of vacuoles, or small holes, in tissue cultures. In my group, Cover had clearly shown that a toxin caused this damage and that it was being made not only by *H. pylori* grown in the laboratory but also by those residing in human hosts. In 1991 we purified the toxin and confirmed Leunk's finding that only 50 to 60 percent of *H. pylori* strains produced it. Our paper was published in May 1992 and included a brief sequence of some of the amino acids that encode for the mature toxin. Based on that scanty information, within the next year four groups—two in the U.S., including our own, one in Italy and one in Germany—were able to clone the gene, which we all agreed to name *vacA*. The race to publish was on. Each of our four papers appeared in separate journals within a three-month period.

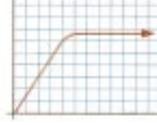
Lest this sounds like duplicated labor, I should point out that each team had in fact solved a different aspect of the problem. We learned, for example, that virtually all *H. pylori* strains possess *vacA*, whether or not they produce the toxin when grown in culture. We also discovered that there is an extraordinary amount of strain-to-strain variability in *vacA* itself. In addition, broth from toxin-producing strains inoculated directly into the stomach of mice brought



DIMITRY SCHIDLOVSKY

***H. PYLORI* INFECTION PROGRESSES to chronic superficial gastritis within months. Left untreated, the condition persists for life in most people. A small fraction, however, may develop peptic ulcer disease, lymphoproliferative diseases or severe chronic atrophic gastritis, leading to adenocarcinoma of the stomach.**

Which Treatment Strategy Should You Choose?

	OLD MODEL	NEW MODEL
CAUSE 	Excess stomach acid eats through tissues and causes inflammation	<i>Helicobacter pylori</i> bacteria secrete toxins and cause inflammation in the stomach, bringing about damage 
TREATMENTS 	Bland diet , including dairy products every hour, small meals, no citrus or spicy foods and no alcohol or caffeine H₂-receptor blockers lessen blood levels of histamine, which increases the production of stomach acid Surgery to remove ulcers that do not respond to medication or that bleed uncontrollably. In the 1970s, it was the most common operation a surgical resident learned. Now it is increasingly rare	Antibiotic regimen. In February 1994 an NIH panel endorsed a two-week course of antibiotics for treating ulcer disease: amoxicillin or tetracycline, metronidazole (Flagyl) and bismuth subsalicylate (Pepto-Bismol). In December 1995 an FDA advisory committee recommended approval of two new four-week treatments, involving clarithromycin (Biaxin) with either omeprazole (Prilosec) or ranitidine bismuth citrate (Tritec). One-week therapies are also highly effective 
SUCCESS 	Patients who stop taking H ₂ -receptor blockers face a 50 percent chance that their ulcers will recur within six months and a 95 percent chance that they will reappear within two years	No recurrence after the underlying bacterial infection is eliminated 
COST 	H ₂ -receptor blockers cost from \$60 to \$100 per month, adding up to thousands of dollars over decades of care. Surgery can cost as much as \$18,000	Less than \$200 for a standard one-week therapy 

ROBERTO OSTI (drawings), LISA BURNETT

about substantial injury. Strains that produce the toxin are some 30 to 40 percent overrepresented in ulcer patients compared with those having gastritis alone. And toxigenic strains usually but not always contain *cagA*, which is located far away from *vacA* on the chromosome.

Slow-Acting Bacteria and Disease

Over the past 15 years, researchers and physicians have learned a good deal about *H. pylori*. This knowledge has revolutionized our understanding of gastritis, formerly thought to repre-

sent the aging stomach, and of peptic ulcer disease and gastric cancer. It has made possible new treatments and screening methods. In addition, a new field of study has emerged—the microbiology and immunology of the human stomach—that will undoubtedly reveal more about persistent infections within mucosal surfaces.

But let us extrapolate from these findings. Consider that slow-acting bacteria, *H. pylori*, cause a chronic inflammatory process, peptic ulcer disease, that was heretofore considered metabolic. And also keep in mind that this infection greatly enhances the risk of neo-

plasms developing, such as adenocarcinomas and lymphomas. It seems reasonable, then, to suggest that persistent microbes may be involved in the etiology of other chronic inflammatory diseases of unknown origin, such as ulcerative colitis, Crohn's disease, sarcoidosis, Wegener's granulomatosis, systemic lupus erythematosus and psoriasis, as well as various neoplasms, including carcinomas of the colon, pancreas and prostate. I believe *H. pylori* are very likely the first in a class of slow-acting bacteria that may well account for a number of perplexing diseases that we are facing today.

The Author

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Further Reading

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