

DENIZENS OF THE DIGESTIVE TRACT:

Aid digestion // protect the immune system // guard against asthma, allergies, perhaps even obesity // exist in an ecosystem that's being knocked **out of balance**.

Our Native Flora

■ BY LAUREN WARE // ILLUSTRATIONS BY MICAH LIBBERG

In 1984, when a young Australian physician named Barry Marshall drank a test tube full of the bacterium *Helicobacter pylori*, he expected bad things to happen. Though many bacteria are harmless or even beneficial, Marshall and his colleague Robin Warren, who in 1979 had gotten the first glimpse of *H. pylori* near areas of inflammation in tissue samples from ulcer patients, had come to believe that this microbe actually caused peptic ulcers. Marshall, in what would make him one of the world's most famous self-experimenters, set out to prove it.

Medical wisdom at the time had another explanation for ulcers—stress and excess acid—and in any case, scientists were certain that bacteria couldn't survive in the stomach's harshly acidic environment. Those views persisted despite findings from as far back as the 1950s that antibiotics were effective against at least some peptic ulcers—indirect evidence that bacteria were present and causing problems. And indeed, within five days of swilling the microbe, Marshall showed classic signs of gastritis: stomach pain, nausea and vomiting, all clinical precursors to peptic ulcer disease.

In the years that followed, Marshall and Warren's hypothesis became widely accepted, and using antibiotics to treat *H. pylori* became standard practice. Yet while the researchers' work led to a cure for a serious disease—and a Nobel Prize in Physiology or Medicine in 2005—it also had an unintended consequence. Because of the now routine purging of this long-term denizen of the human stomach, the percentage of people in developed countries who culture positive for

H. pylori has decreased dramatically, while new diseases of the digestive tract—gastroesophageal reflux disease, or GERD; and esophageal adenocarcinoma, a deadly form of cancer—have emerged as if from nowhere.

The disappearance of *H. pylori* is a significant loss, asserts Martin Blaser, a microbiologist at New York University Langone Medical Center, who, like Marshall, has had to battle accepted scientific wisdom. “When I began to publish about *Helicobacter* 12 or 13 years ago and said that it was becoming extinct, it was almost heresy,” Blaser says. “But if an ancient organism goes extinct, it's going to have consequences.”

Blaser's argument could apply to many other microbes that also seem to be disappearing just as we begin to understand that the bacteria that inhabit us—our microbiota, which outnumber human cells 10 to one—are an intrinsic part of our health. Resident microbes help extract energy from food, stimulate the immune system and provide a buffer against invading pathogens, among other important tasks. Only recently have scientists managed to link imbalances in our microbial makeup to cancer, inflammatory bowel disease and obesity. And though we harbor populations of bacteria in and on many parts of our bodies, those in our digestive system have been studied in the greatest depth, partly because they are the most numerous and partly because it's suspected that gut microbiota have the most intimate connections to overall human physiology.

But those connections are in flux, as changes in lifestyle and medical practices during the past century—particularly the widespread use of antibiotics—have put pressure on many human-dwelling bacteria. “We've rapidly changed the treaty





that we established with our microbes over a very, very long time,” says Betsy Foxman, a molecular epidemiologist at the University of Michigan School of Public Health in Ann Arbor. That treaty: We would protect our microbes from extinction by giving them shelter and food, and they would contribute to our health. But we’re no longer protecting them.

Since the emergence of sponges some 500 million years ago, animals have coexisted with microorganisms. In humans, they colonize from head to toe, forming complex ecological communities, interacting with one another and with human cells. They are extremely varied, with scientists identifying more than 1,000 species belonging to more than 50 different phyla, or “tribes.” Although most are bacteria, a few are fungi, viruses and other organisms called protists, which resist easy classification. So far, we know that these organisms synthesize vitamins; stimulate development of tissues, including parts of the gastrointestinal tract, the cecum and some lymphatic tissues; and protect us from pathogenic bacteria by competing with them for nutrients and attachment sites, by stimulating production of antibodies and, possibly, by secreting substances that destroy or inhibit certain pathogens.

With growing knowledge about the microbes in and on people have come key tenets defining the microbes’ relationship with human hosts. They have evolved alongside us, with ongoing natural selection for bacteria that benefit both humans and their own species. And some microbes colonize specific parts of the body. *E. coli* inhabit the colon, lactobacilli colonize the vagina, and staphylococci live on the skin. Researchers often refer to resident microbiota as commensals, meaning that their presence doesn’t harm us—the word, from Latin, means “eating at the same table.” But research has revealed that a single bacterium can have both beneficial and harmful effects. For example, although in most people *H. pylori* has lived as a commensal, in some it creates a painful, damaging stomach ulcer.

Yet just as scientists begin to skim the surface of what humans’ resident bacteria are and what they might be doing, they’re also finding that the bugs are changing—sometimes dramatically, as in the case of *H. pylori*. To Blaser and others, its rapid disappearance seems to be linked to major changes in human ecology. As Blaser and his colleague Stanley Falkow,

For our microbes, changes in human behavior during the past few generations amount to a threat to their existence and a reason to evolve new defenses that may have unhappy consequences for their human hosts.

a professor of microbiology and immunology at Stanford University School of Medicine, laid out in an essay in *Nature Reviews Microbiology*, during the past few generations we’ve limited our bacterial exposure by living in smaller families, having less contact with other people (contact that used to come via shared beds, closer living quarters and communal food) and consuming cleaner water and food. Increasingly common cesarean section births deprive babies of the chance to be colonized with their mothers’ bacteria as they pass through the birth canal; meanwhile, breast-feeding—another rich source of microbes—significantly declined during the first half of the 20th century, though it has rebounded somewhat since the 1970s. And antibiotics, particularly in children, can wipe out swaths of microbial residents with one course of treatment.

These changes amount to a threat to our microbes’ existence and a reason to evolve new defenses that may have unhappy consequences for their human hosts. “They’ve adapted,” presumably by changing their genes, says Foxman, “because they live and evolve on such a short life span—producing new generations in minutes or days—while our genes have not.”

Blaser and other researchers have discovered that certain strains of *H. pylori* are more likely than others to cause inflammation in stomach cells. Attempting to regulate the



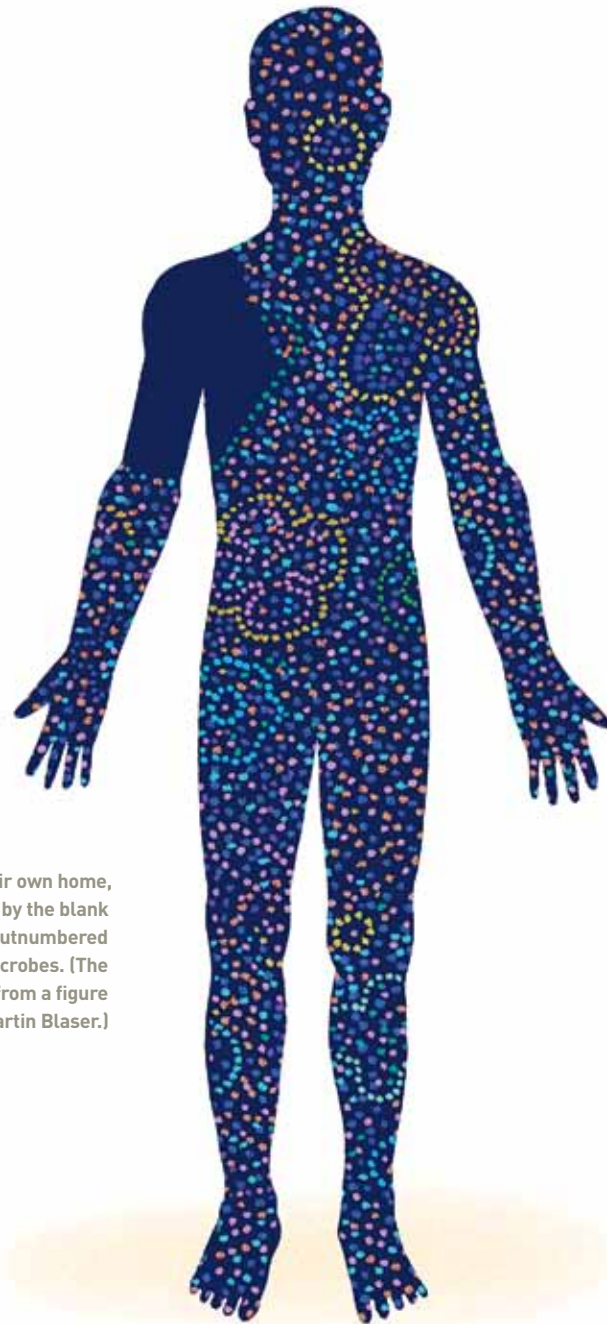
stomach's acidity to more comfortable levels for their own survival, *H. pylori* strains that bear a gene called *cagA*—which is responsible for the CagA protein—also contain genes that encode a type IV secretion system (TFSS), a group of proteins that export large, complex molecules into host cells.

Research has shown that *cagA*-bearing *H. pylori* strains create TFSS proteins that form a structure somewhat like a microscopic hypodermic needle. This structure injects the CagA protein into the epithelial cells lining the stomach. Enzymes in human cells then change CagA's chemistry, which affects signaling mechanisms in those cells. This allows CagA to interact with human proteins and cause the release of inflammatory proteins called cytokines—part of the innate immune system, the first line of defense against infection—which in turn attract neutrophils, white blood cells that damage the stomach lining by releasing free radicals (highly reactive oxygen and nitrogen compounds). The resulting inflammation affects acidity in the stomach by influencing the hormonal regulation of acid-producing cells. *H. pylori* interacts with its host in a feedback loop, regulating the level of acidity in the stomach and keeping it in balance so that the bacterium can exist in the extremely acidic stomach.

GERD, a chronic disorder involving the regurgitation of acidic stomach contents into the esophagus, is associated with a lack of *H. pylori*, possibly because the absence of the bacterium allows the stomach's acidity levels to go unchecked. On the flip side, the presence of a CagA-producing strain of *H. pylori* also increases the risk of stomach cancer, owing to those needlelike structures. Over time they can damage the cells lining the stomach.

As *H. pylori* has disappeared, the incidence of adenocarcinoma of the esophagus, which was rare, has sharply increased, and a diagnosis of GERD turns out to be a primary risk factor for the cancer. A study by Blaser and his colleagues found that people carrying CagA-producing strains of *H. pylori* were at significantly lower risk of developing these cancers and of having GERD or a precancerous lesion called Barrett's esophagus. Additional studies by independent researchers confirmed the correlations, though scientists are still trying to untangle the web of *H. pylori*'s beneficial and harmful effects.

Some of the bug's positive impact extends beyond the stomach to other bodily systems. Recent epidemiological studies have connected *H. pylori*-positive status, and specifically the presence of CagA-producing strains, to a reduced risk of developing childhood asthma, skin allergies and allergic rhinitis. This makes sense, because previous studies have found



A distinct minority in their own home, human cells—represented by the blank shoulder area—are outnumbered 10 to one by resident microbes. (The illustration is adapted from a figure created by microbiologist Martin Blaser.)

that people harboring *H. pylori* have higher populations of immune system T cells in the stomach, including a subset of those infection-fighting cells that regulate immune function throughout the body. In people without *H. pylori*, there are far fewer immune cells in the stomach.

Research has also shown that when antibiotics kill *H. pylori*, there is a decrease in leptin, a hormone that signals the brain to stop eating, and an increase in ghrelin, a hormone that stimulates the appetite. One study demonstrated that patients given antibiotics to eradicate *H. pylori* gained more weight than those who had not had the treatment. Is it possible that the

Microbes on the Menu? //

People receive prescriptions for them and even buy foods filled with them, purportedly to restore the natural balance of their gut microbiota. But the jury is still out on how, and how well, probiotics work.

Probiotics have been around for thousands of years, in traditional fermented foods such as yogurt, sauerkraut and kefir, all of which teem with colonies of microbes. Yet probiotics—by the World Health Organization’s definition, “live microorganisms which, when administered in adequate amounts, confer a health benefit on the host”—have moved from the fringes to the mainstream only in the past decade or so.

A walk down the supermarket dairy aisle reveals an array of yogurts and dairy-based drinks containing “live and active cultures” of bacteria that supposedly bolster health and digestion. In the supplements aisle, consumers

can choose from capsules, powders and liquids that contain a wide variety of microbes. But what effect do they really have on our health?

The global probiotics market, which yielded \$15.9 billion in 2008, is expected to nearly double by 2014, thanks in part to the many physicians who regularly recommend probiotic supplements during and after a course of antibiotics to help prevent the diarrhea that sometimes accompanies antibiotic-induced die-off of normal gut microbes. Some studies have shown that certain strains of commercially available probiotics positively affect immune response and can protect against infection and

diarrhea. Most of the studies, however, have been small, and many have been conducted on mice, so scientists caution against drawing blanket conclusions about probiotics’ effects. And while probiotics don’t have side effects for most people, they can exacerbate problems in certain groups of patients.

Though researchers agree that probiotics likely help in some way, they emphasize that we’re far from understanding their mechanisms. Researchers hope that the advent of more advanced genetic sequencing technology will shed light on what happens to our gut microbiota when we ingest probiotics.

disappearance of *H. pylori* is also connected to the rise of obesity in recent decades? “Whether this is going to be a big or small piece of the obesity story, I don’t know,” says Blaser. “But our physiology is changing.”

Charting alterations in *H. pylori* and other human-dwelling microbes has been aided by the ability to sequence large amounts of microbial 16S rRNA, a sort of bacterial bar code that identifies each species. Recent work has provided a much fuller picture of which species are present in the gut and in what proportions and has revealed connections between changes in other gut-dwelling microbes and rising rates of obesity and metabolic syndrome.

Two major initiatives should now further expand our understanding of what bacterial species exist in our bodies, what they’re doing there and how they may differ from one person to the next. By cataloguing the microbial genome, or microbiome, the Human Microbiome Project, launched by the National Institutes of Health in 2007, attempts to answer those questions. A similar undertaking financed by the European Commission, called MetaHIT, has the same goal but focuses specifically on the human intestinal tract.

The first results from the MetaHIT project, published in March in *Nature*, consist of a census of the microbial genes,

collected via fecal samples, that are present in the human digestive system. The number topped out at 3.3 million genes, representing approximately 1,150 species, with 57 species shared among more than 90% of individuals. This suggests there may be a core set of microbes that most people carry. But there was also a wide variety in the relative abundance of the most common species from person to person. The sheer number of genes suggests a greater microbial diversity than scientists had predicted, and it dwarfs the human genome, which consists of roughly 20,000 genes.

The Human Microbiome Project encompasses studies in which researchers aim to develop a reference collection of microbial genomes. In the May 21, 2010 issue of *Science*, the HMP published an analysis of 178 of them—the beginning of a collection that may eventually include 900 species. In the project’s second phase, studies will attempt to associate specific microorganisms with states of health and illness. Another main goal is to develop tools to advance the field of metagenomics, the analysis of genetic material from a mixed community of organisms. This will let researchers look in more detail at how microbes interact with human physiology.

Other research is already pulling back the curtain. A team led by Andrew Gewirtz, an immunologist at Emory University School of Medicine in Atlanta, studied a group of mice genetically engineered to be without a protein called Toll-like receptor 5, which is



normally found in the intestines. TLR5's function is to recognize the protein flagellin, which makes up the tails that bacteria use to move. Detecting flagellin from an invading pathogen, the protein mobilizes production of cytokines. A tenth of the mice lacking TLR5 developed severe colitis, while 30% showed less severe colitis, and the remainder exhibited overall inflammation and higher weight. To rule out the possibility that the inflammation was caused by an infection, Gewirtz then "re-derived" the mice, a process by which embryos from the genetically altered mice are transplanted into germ-free mothers.

Gewirtz and his colleagues found that the re-derived TLR5-deficient mice began to overeat; became obese; and developed high blood pressure, high cholesterol and insulin resistance—all key features of metabolic syndrome. Those changes occurred alongside alterations in the gut microbiota of the mice. When their intestinal bacteria were transplanted into typical mice with the TLR5 gene but no existing microbiota, the new mice developed many of the same symptoms—suggesting that the change in the composition of the gut microbiota, rather than the lack of the TLR5 protein, leads to metabolic syndrome.

"The TLR5-deficient mice had chronic intestinal inflammation," Gewirtz says. "We suspect that can result from the inability to manage the gut microbiota properly." Though the precise mechanisms are not yet known, Gewirtz concludes that the lack of TLR5 causes changes in the balance of intestinal microbes, which may lead to low-grade inflammation. That desensitizes the body's response to insulin, a hormone that regulates the amount of glucose in the blood, which in turn causes a tendency to overeat and ultimately results in metabolic syndrome.

Gewirtz's results jibe with other work on obesity, intestinal diseases and gut microbes. A 2006 study by Peter Turnbaugh, Jeffrey Gordon and colleagues at the Center for Genome Sciences at Washington University School of Medicine in St. Louis showed that obesity in mice seemed to rise alongside a shift in the relative abundance of the two dominant phyla of bacteria in the gut, with the level of Bacteroidetes being reduced by half and Firmicutes increasing by the same proportion. Researchers have discovered the same alteration in obese humans. After the microbiota of the obese mice were transferred to germ-free mice, they gained more fat than did recipients of microbiota from lean animals, reinforcing the notion that the change in gut microbiota may contribute to obesity. "The trend toward overeating may not simply reflect reduced willpower or greater availability of food," says Gewirtz. "There may be a biological change driving it."

Such insights may proliferate as researchers learn more about the identities and roles of the microbes that live with humans and how they interact with one another, with us and with the environment. A growing understanding of the human microbiota could eventually lead to new therapies, suggests Justin Sonnenburg, a microbiologist at the Stanford School of Medicine. He envisions drugs that would target a microbe or a group of microbes in some as yet unknown way and alter a patient's microbiota to promote better health. "It's something that holds great potential," Sonnenburg says. It's a concept he and his research group are actively pursuing.

But we're just scratching the surface of understanding our microbiota as an ecological system. The next steps for the Human Microbiome Project and MetaHIT are to look for microbial differences between healthy individuals and those who have specific diseases. They'll also begin to develop tools to analyze the massive amounts of genomic data in more detail, to try to increase our knowledge of the metabolic interactions between microbe and host. "These projects will make it possible to really understand in a logical way what types of bacteria correlate with metabolic health," says Gewirtz. "The results might be analogous to what has happened with the human genome—there won't be any miracle cures at the moment it's completed, but the information will be essential."

What is already clear is the vast complexity of the microbes that live within us and how intricately linked they are to our own biological processes. To paraphrase Walt Whitman, we are large, we contain multitudes. ■



→ DOSSIER

1. "What Are the Consequences of the Disappearing Human Microbiota?" by Martin J. Blaser and Stanley Falkow, *Nature Reviews Microbiology*, Nov. 9, 2009. The authors argue that our ancestral indigenous microbes, intricately linked to our physiology, are disappearing and that the consequences may include the rise of modern diseases such as asthma and obesity.
2. "A Catalog of Reference Genomes From the Human Microbiome," by the Human Microbiome Jumpstart Reference Strains Consortium, *Science*, May 21, 2010. In this groundbreaking study by the Human Microbiome Project, the authors sequenced nearly all the genes of 178 microbial species from various parts of the body.
3. "Transfer of Carbohydrate-Active Enzymes From Marine Bacteria to Japanese Gut Microbiota," by Jan-Hendrik Hehemann et al., *Nature*, Apr. 8, 2010. This study demonstrates that Japanese subjects' gut microbiota acquired genes from a marine bacterium, allowing the microbes to digest components of their hosts' regular diet: seaweed.