

While we tend to think of bacteria as harmful, we all carry plenty of microbes that work to the good. Can we use them to prevent or treat diseases?

By Maya Pines

The Friendly Bacteria Within Us

THE NIGHT BEFORE

having dental surgery in 1998, a 71-year-old Canadian woman was given antibiotics to prevent infection. The operation on her teeth went well, but a few days later she developed diarrhea so severe that she went into shock and was rushed to the hospital. Tests showed she had been hit with toxins produced by *Clostridium difficile*, a generally mild bug that resides naturally in the intestinal tract. Usually kept in check by the body's "good" bacteria, *C. difficile* poses little threat unless something—like a course of antibiotics—kills off some of those protective bacteria.

After two months of intensive treatment and physical rehabilitation, the dental patient survived. Other people have not been so lucky. In a single Quebec hospital over the last 18 months, 100 patients died of *C. difficile* infection. Fatalities of this sort have been increasing rapidly not only in the province of Quebec, whose health minister suggested that "enthusias-

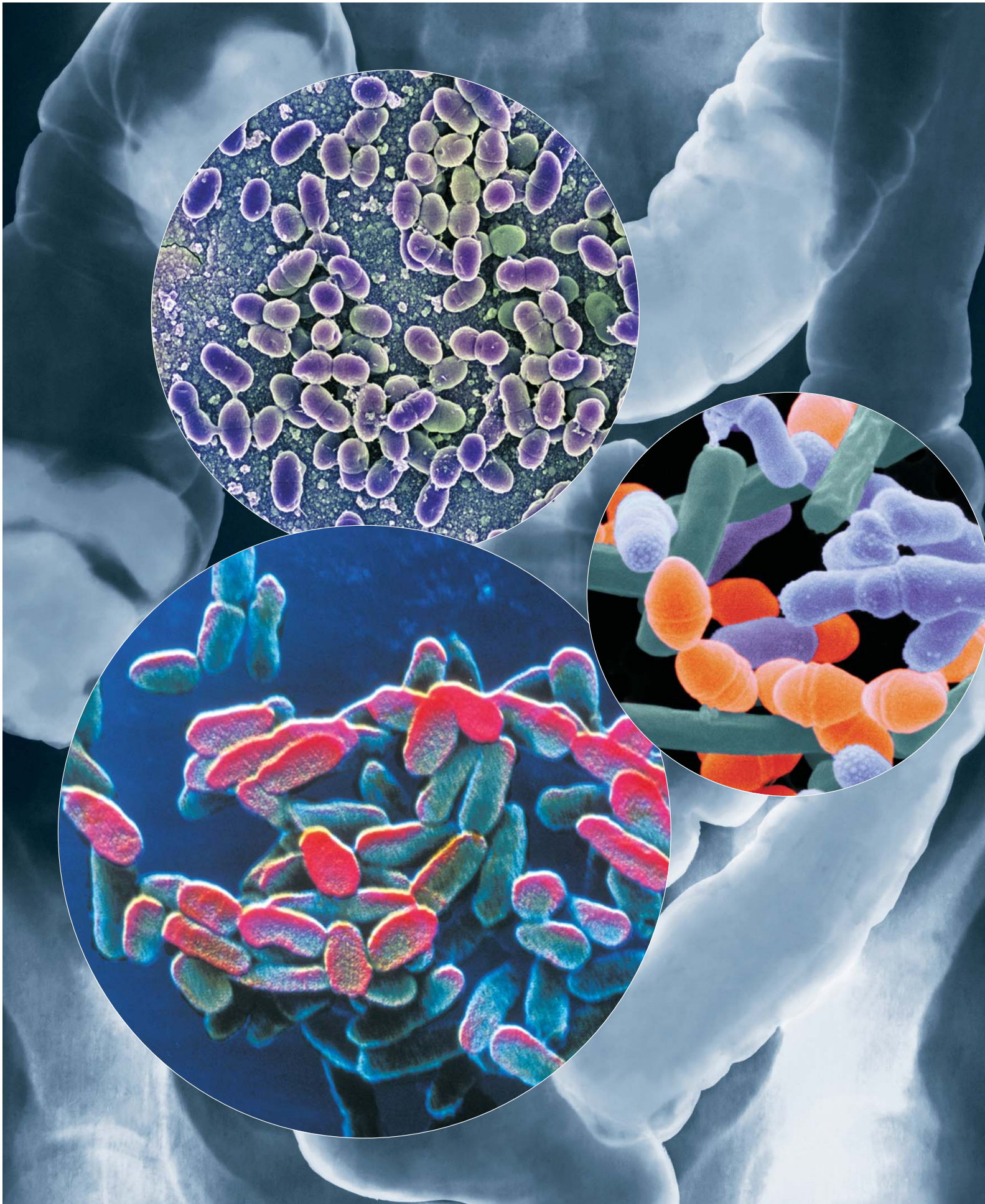
Beneficial bacteria include (top) *Lactococcus lactis*, as well as (right) *Lactobacillus bulgaricus* (blue), *Streptococcus thermophilus* (orange), and a member of the *Bifidobacterium* family (magenta), all found in yogurt and cheese. Normally benign, *Bacteroides fragilis* (bottom) is an intestinal microbe that can wreak havoc under certain conditions, such as post-surgery. (The bacteria in these scanning electron micrographs have been color enhanced.)

tic" prescribing of antibiotics might have caused the outbreaks, but in other parts of Canada and the United States as well.

We each carry two to five pounds of live bacteria in our bodies. Some, like *C. difficile*, are potentially harmful. Many bacteria, however, are quite useful—so useful, in fact, that we could not live without them.

Until recently, scientific research has focused on fighting "bad" bacteria—the ones that cause cholera, scarlet fever, typhoid, tuberculosis, and other major infectious diseases. Scientists pretty much ignored the good bacteria, which often outnumber the bad ones.

In the past few years, however, researchers have begun to recognize the enormous contributions made by this friendly "bacterial nation," as Jeffrey I. Gordon, director of the Center for Genome Sciences at Washington University School of Medicine in St. Louis, calls it. Trillions upon trillions of



microbes, representing some 1,000 species, are packed within us, especially in our guts. A single milliliter of the colon's contents might harbor 100 billion of them. This "nation" functions like a kind of internal organ, says Gordon, affecting our well-being.

THE PROMISE OF PROBIOTICS?

Using good bacteria to promote health—a practice sometimes called probiotics—has a long history, but it never quite became an accepted therapy. About a century ago Elie Metchnikoff, director of the Pasteur Institute in Paris, France, started a yogurt craze when he announced that Bulgarian peasants who ate yogurt regularly tended to live to ripe old ages; yogurt contains live cultures of lactobacilli, one of the better-known strains of good bacteria. Several other substances that supposedly contained beneficial bacteria were also used to treat infections in the gut or vagina. This practice ended around the time of World War II, when the newly discovered, often life-saving, antibiotics proved to work more rapidly and effectively.

Now, however, "We are being forced to look at alternatives to antibiotics to combat the ever-increasing number of infections that occur because of excessive use of antibiotics," Christopher J. Bulpitt and his colleagues at London's Imperial College School of Medicine wrote in the *British Medical Journal* in 2002. Severe diarrhea, for instance, often results from treatment with antibiotics, which wipe out good bacteria along with the bad. Could this side effect be prevented by maintaining enough good bacteria in the patients' intestines to act as guardians?

To find out, Bulpitt's team analyzed nine randomized, double-blind, placebo-controlled trials in which all patients had been treated with antibiotics, but some also received various combinations of microbes that were believed to be good, while others received placebos. The team concluded that "probiotics are a possible solution," but only for preventing antibiotic-associated

diarrhea. They found little support for using probiotics as a cure.

One way to learn which bacteria are most useful in particular circumstances is to let nature be your guide, as a group of Swedish researchers did when they concocted a highly unusual nasal spray. In 2001, Kristian Roos and his associates at the Lundby Hospital in Gothenburg were seeking new treatments for infants and toddlers who have repeated bouts of ear infections (otitis media). They targeted the one child in 20 who comes down with these painful infections frequently—up to six times a year—despite repeated treatment with antibiotics, to which the child becomes increasingly resistant. So they started out by "harvesting" the microbes they found living in the eustachian tubes (which connect the nose and middle ear) of healthy children at a daycare center. Among these microorganisms they identified some 800 different strains of α -hemolytic streptococci. Next they tested each strain's ability to stop the growth of otitis-causing bacteria in the lab. Finally, they chose the five most active strains, which they put into the nasal spray.

At the end of three months, 42 percent of the children who had been given this bacterial spray remained free from otitis, while only 22 percent of those who received a placebo escaped new ear infections. The scientists concluded that "recolonization" with selected bacteria does protect against recurrent attacks of otitis, at least to some extent.

THIN LINE

The British and Swedish efforts, and other clinical studies of this sort, generally paint an optimistic picture of probiotics. But many scientists remain skeptical, and few such treatments are currently in use. The real hurdle is still our lack of precise knowledge. What proportion of the bacteria in our bodies is good? How many are pathogenic? How many good bacteria sometimes become bad, and vice versa? Are many of them simply straddling the fence?

Nobody knows. An adult human has about 10 times more microbial cells than human cells, so "based on cell number, each of us is 90 percent microbial and 10 percent human," says Gordon. "The genomes of our gut microbes probably contain 100 times more genes than our own genome, providing us with traits we haven't needed to develop on our own." Yet at least half of these bacteria cannot be grown outside the gut because "we haven't learned how to reproduce their normal conditions in the lab," he says, "so we don't have an accurate view of them." Together with some acquired viruses, yeast cells, archaea (single-celled microorganisms that live in geysers and other extreme environments), and occasional parasites, the bacteria form "a constantly open ecosystem," Gordon says.

Some of the good bacteria have a symbiotic relationship with our intestines (they help us and we help them, usually by providing nutrients). Others have a commensal relationship (one partner benefits without harming the other). But in our guts, nothing is permanent. Bacteria take on shifting roles as they encounter changing circumstances. "If the formidable barrier pro-

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What Good Bacteria Do

Some things we know about good bacteria, besides the generalization that they help to counteract pathogens:

- ▶ Good bacteria can break down certain foods, such as plant starches, that we cannot digest on our own. "This enables us to extract more energy from what we consume," says Jeffrey I. Gordon, director of the Center for Genome Sciences at Washington University School of Medicine in St. Louis. (Similarly, cows can digest cellulose thanks to the good bacteria that live in their rumens.)
- ▶ Good bacteria promote the storage of energy as fat. According to Gordon, this raises the possibility that "an individual's predisposition to obesity or leanness may be partly determined by the composition of the microbes living in the gut."
- ▶ Good bacteria help shape our postnatal development. For example, they help to form our intestinal blood vessels, through which we absorb nutrients.
- ▶ Good bacteria synthesize vitamin K and other vitamins that we cannot generate on our own. They break down carcinogens. They also may influence the metabolism of drugs.
- ▶ Good bacteria increase the rate at which the cells of the intestinal lining renew themselves, ridding us of damaged cells that could bring on gastrointestinal cancer.
- ▶ The good bacteria that infants acquire from their mothers and from the general environment at birth "educate the newborns' immune systems," says Gordon. "This appears to reduce allergic responses."
- ▶ Each human carries a different set of bacteria, and its composition varies along the length of the gut. Some of these bacteria are permanent residents; others are transient "tourists," just passing through.



Jeffrey I. Gordon (left) and Eduardo A. Groisman, both at Washington University School of Medicine in St. Louis, are among investigators who study bacteria—both the “good” and the bad—that comprise what Gordon calls the “bacterial nation.”

duced by symbiotic bacteria is destroyed,” notes Gordon, “some previously minor bacteria can expand and produce disease. There’s also a lot of horizontal gene transfer (from one bacterium to another), creating new strains and spreading antibiotic resistance. It’s very dynamic!”

“Take *Bacteroides fragilis*, for instance. Usually it’s fairly innocent,” he says. “But after stomach surgery or some other insults, it can cause abscesses. A researcher at Harvard, Laurie F. Comstock, recently discovered that this happens when the bacterium’s outer capsule changes, making it more dangerous.”

Similarly, up to two-thirds of the world’s population carries *Helicobacter pylori*, and in most people it does no harm. In 10 percent of infected people, however, it leads to stomach ulcers or cancer (which may be either gastric lymphoma or adenocarcinoma of the stomach).

“The question one should ask is not how many bacteria in our guts are pathogenic,” says Gordon, “but how many of them have pathogenic potential.”

ORIGINS OF VIRULENCE

Bacterial virulence seems to involve what B. Brett Finlay, an HHMI international research scholar at the University of British Columbia in Vancouver, calls a kind of “cross-talk” between bacteria and their hosts.

Bacteria appeared in the world long before humans did. After we came on the scene, some bacteria “co-evolved” with us so they could take advantage of what is for them a wonderful environment—the human gut, where so many nutrients are concentrated. This meant the bacteria had to learn how to overcome the many physical, cellular, and molecular barriers the human body presented, wrote Howard Ochman, a biochemist at the University of Arizona, in a recent issue of *Science*. They may have added or subtracted certain genes.

Natural selection favored those bacteria that made the most effective changes. This happened “regardless of whether the ultimate outcome of the interaction is harmful, benign, or beneficial to the host,” said Ochman. “Only from the host’s perspective are these distinctions crucial.”

The first job of infectious bacteria is to attach themselves to specific receptors on human cells, says Finlay. And sometimes the host cell collaborates. As his team discovered while studying diarrhea-causing enteropath-

ogenic *Escherichia coli* (EPEC), these bacteria use two different kinds of adhesive molecules to latch onto human cells. The first molecule somehow “rings a doorbell” on the host cell, telling it to produce a sort of pedestal, which almost immediately grows out of the cell surface. This pedestal then enables EPEC to attach itself securely to the cell with its second adhesive molecule.

Although most diseases are caused by the initial adherence of bacteria to cells, says Finlay, no drugs are yet available to derail this process. If scientists learn to block bacterial attachment, they may be able to prevent or stop infections, he suggests.

But why these particular bacteria tried to adhere to human cells in the first place remains a mystery. Were they previously good bacteria that somehow turned bad?

Eduardo A. Groisman, an HHMI investigator at Washington University’s School of Medicine in St. Louis, first tackled this problem 10 years ago in an article, “How to become a pathogen,” that he and Howard Ochman published in *Trends in Microbiology*. The idea was to find the genes responsible for producing virulence, focusing on differences between the activities of nonpathogenic *E. coli* bacteria and a strain of *Salmonella* that can cause typhoid fever. With the tools available at the time, and more recently with the help of sequenced microbe genomes, the two scientists worked to identify “pathogenicity islands”—groups of genes that are found only in pathogens and that contribute to disease.

In some cases, however, pathogenic and benign bacteria have almost the same genes, Groisman observes. One possible explanation for their different behavior is that these genes are regulated in alternative ways. “We’re now studying how this differential regulation may affect virulence,” he says.

ANIMAL MODELS

The complexity of the interactions between the huge bacterial nation and the human gut “defies imagination,” says Gordon. So his lab created animal models that could be analyzed more easily. All the animals—mice or zebrafish—were raised under germ-free conditions; for comparison, half were later “colonized” with specific strains of bacteria.

A Palliative for Chemo?

HHMI investigator Ruslan Medzhitov, an immunologist at Yale University School of Medicine, recently discovered an entirely new role for our resident bacteria: They help protect us from radiation and poisons. As he explained in a paper in *Cell* published July 23, 2004, he came to this conclusion indirectly while studying the cells of the innate immune system, our first line of defense against pathogens. Medzhitov expected that the receptors on these cells would detect only the bad microbes. "We thought these receptors would simply ignore any components of good bacteria," he says. "But on the contrary, they recognized both good and bad." As he later found, it was absolutely essential for these immune-system cells to recognize good bacteria to stimulate the intestinal tissue's systems of maintenance and repair.

In one experiment, Medzhitov's team worked with three groups of mice that were exposed to fairly strong radiation, as much as might be used to kill tumor cells.

One group, which had normal bacterial "flora" in their guts, survived the radiation with relative ease. When the team irradiated a second group of mice, whose colons had been deprived of normal bacteria by antibiotics, they all died. Then the researchers tried to prevent such deaths by giving a third group of mice—similarly deprived of normal bacteria—a chance to drink water that had been laced with some components of good bacteria. Although they received the same dose of radiation as the second group during that time (one week), they all survived. "The cells of the mice's innate immune systems recognized the bacterial components they had swallowed," says Medzhitov. "That activated their immune systems and protected the mice from some of the damage they would otherwise have suffered." The team obtained similar results in mice exposed to toxic chemicals.

This finding raises the hope that human cancer patients may be protected in similar ways. Restor-



ing the normal supply of good bacteria in their intestines might help them avoid a possible side effect of radiation or chemotherapy: an injured and bleeding gut, which can be fatal.

For the mouse experiments, Gordon chose a strain of *Bacteroides thetaiotaomicron*, good bacteria frequently found in the guts of both mice and humans; its main job is to provide the enzymes needed to process certain carbohydrates in plants. The *B. thetaiotaomicron* genome was sequenced in 2003 and the proteins it produces have been sorted out, making it possible to examine the microbe's activities with some of the newest tools of genetics. The Gordon team has shown, for instance, that *B. thetaiotaomicron* stimulates production of an antibiotic protein that can kill infectious microbes such as *Listeria monocytogenes*, which causes food-borne gastroenteritis. Another of the researchers' findings is that *B. thetaiotaomicron* promotes the development of small "networks of branched, interconnected blood vessels" in newborns, the scientists reported in the *Proceedings of the National Academy of Sciences*. This capillary network does not grow properly in germ-free mice.

Zebrafish offer several advantages to researchers. Because these small fish develop rapidly (the larvae hatch within three days of fertilization) and remain transparent through early adulthood, it is possible to observe the embryos' growing digestive tracts and their resident bacteria. Using DNA microarrays, Gordon's team recently examined the genes that were activated in zebrafish intestines after exposure to specific good bacteria. They found that 59 zebrafish genes responded to these bacteria in exactly the same way as do the corresponding genes in mice, even though the two species diverged millions of years ago. This implies that responses to these bacteria go back very far in evolution; most likely they were critical not only to mice and zebrafish but also to many other species.

OVERSOLD THERAPIES?

While researchers work step by step, accumulating information with the aid of animal models, the marketplace apparently is not waiting for final results. Various brands of probiotic food supplements are already being sold around the world with promises of fabulous benefits for cats, dogs, birds, horses, and farm animals: "improved growth," "better health," "establishment of beneficial gut microflora," "better utilization of food," "reduced intestinal upsets," and "increased resistance to infections," which should reduce the

need to treat livestock with antibiotics.

Chickens in particular are frequently treated with Preempt, a product developed with help from U.S. Department of Agriculture scientists, which contains 29 kinds of good bacteria found in healthy chickens. At least 10 percent of chickens are infected with *Salmonella* bacteria, a leading cause of food-borne illnesses in humans. The idea is to spray newly hatched chicks with Preempt so that when they peck at their wet feathers they will swallow its bacteria. The reasoning is that the product's good bacteria will grow in the chicks' intestines, forming a protective barrier that cannot be breached. As a result, any ingested *Salmonella* will be unable to attach themselves to the chicks' intestines and will be forced out of the animals' bodies. This model seems to work to some extent.

When it comes to humans, however, the use of probiotics remains more controversial. There are strong commercial interests in its favor—yogurt marketed as "probiotic" is one example—but inconsistent experimental results. Much of the published research consists of reports on only a few patients, and many of these reports are contradictory. Skeptical scientists have called probiotics everything from "conbiotics" to "snake oil." Even the manufacturers of probiotic compounds agree that more precise information is needed.

According to the *Harvard Health Letter* of March 2002, "Probiotics have been oversold. The claims are seductive: pills, powders, and solutions containing 'friendly' bacteria will boost the immune system, prevent cancer, and perform assorted other health miracles. ... But that doesn't mean it's based upon total fiction. ... The evidence suggests that probiotic therapy could be useful someday as a form of preventive medicine—and not just for diseases affecting the gut."

As Gordon puts it, "Bacteria have learned to manipulate our biology in many ways that benefit themselves and us. We now have the tools to identify the pathways through which they operate, as well as the chemicals they synthesize." This information could lead to new ways of diagnosing, treating, and ultimately preventing a variety of diseases. "Bacteria are fabulous teachers," says Gordon. "They are pointing the way." **H**