

THE GOOD, THE BAD, AND THE UNKNOWN:

BACTERIA IN YOUR BODY

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INSIDE YOU RIGHT NOW THERE ARE MORE BACTERIAL CELLS THAN HUMAN CELLS. IN FACT, IF YOU CRACKED OPEN EVERY CELL IN YOUR BODY, UNWOUND THE DNA, AND STITCHED IT TOGETHER, YOU'D FIND TEN TO A HUNDRED TIMES MORE BACTERIAL DNA THAN HUMAN DNA.

In our mouths, on our skin, and in our digestive and reproductive tracts, bacteria have been with us since the beginning, evolving with us. We know some of them. Researchers identified *Lactobacillus acidophilus* many years ago. You might know it from reading yogurt labels. It's supposed to be good for us. *E. coli* live inside us. We know a lot about them. They're not so good.

Most of the bacteria in our intestines, though, can't survive exposure to oxygen, which means they can't be cultured on a plate and studied like other bacteria. Scientists estimate that there are hundreds, maybe thousands, of species inside us, but we know little about them because we haven't been able to study them—until now.

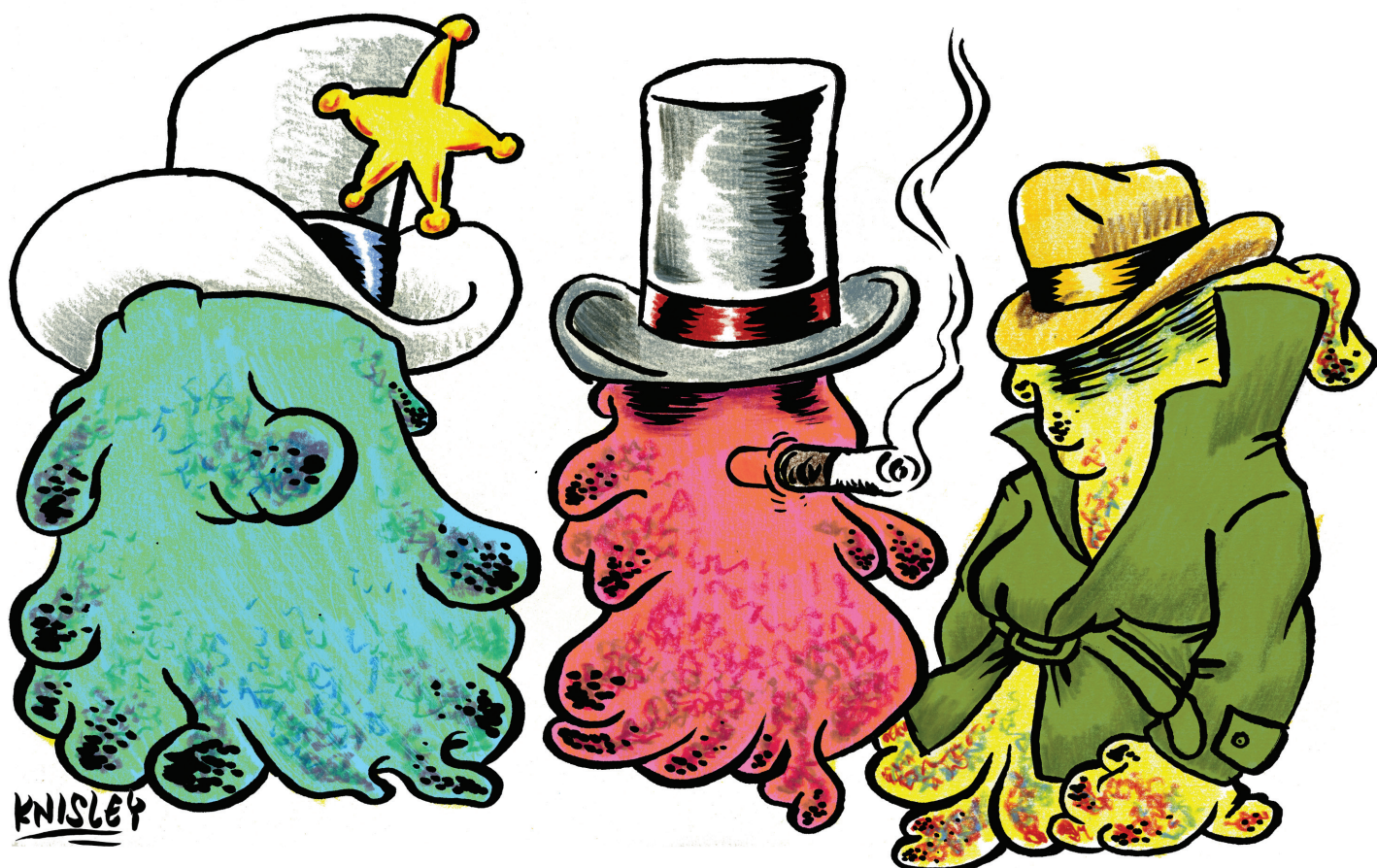
As part of the Human Microbiome Project, which is a National Institutes of Health mandate to identify and characterize the bacteria that call humans home, scientists use new methods that allow them to isolate and sequence bacterial DNA. This means they are finally identifying more strains of bacteria. At UNC, some of these new technologies are housed in the Microbiome Core Facility, where researchers can figure out the bacterial

composition of, well, anything. And there are plenty of reasons why we want to figure out what bacteria are doing inside of us, especially in our bellies.

For centuries scientists didn't think our relationship with bacteria was terribly important, except in those rare cases when bugs escape the GI tract, enter the bloodstream, and cause a life-threatening infection. Then researchers started figuring out that our intestines are full of known and unknown species that compose unique bacterial ecologies. And each of us, they're finding, has a symbiotic relationship with these bacteria.

Today we're learning that diverse groups of bacteria play major roles in several ailments, including colitis, Crohn's disease, irritable bowel syndrome, obesity, diabetes, cystic fibrosis, and even colon cancer. Scientists at Carolina and elsewhere are proving that probiotics—the so-called good bacteria sold at health-food stores—can help millions of people, including kids. And they've found that bacteria's effect on health starts when we're babies.

In the following pages, UNC researchers tell of their findings even as they continue to explore uncharted territory—inside us.



ON THE ATTACK

What triggers the inflammation that causes gut problems?

Balfour Sartor couldn't shake the fevers. It was 1971 and he was a college senior. He had lost weight, felt tired all the time, and had no idea why. His doctor came up with an unusual diagnosis: Crohn's disease, a serious inflammation of the small intestine. "I had never heard of it," Sartor says. "The doctor gave me anti-inflammatory pills and steroids, totally nonspecific medications that dampen the immune system response."

The doctor knew that an overactive immune system could trigger inflammation, but he didn't know what caused Sartor's T-cells and B-cells to attack the walls of his small intestine. No other doctor did, either—a fact that inspired Sartor to specialize in gastrointestinal disorders during medical school. He became a GI fellow at UNC.

One day Sartor ran into his boss, who handed him a journal article. "It was about how a purified bacterial product caused arthritis in rats," Sartor recalls. The bacterial product was a polymer that's found in the cell walls of virtually all bacteria. Immediately, Sartor had an idea. Arthritis, essentially, is a kind of inflammation. Bacteria live in our digestive tracts and are in their highest concentrations in the parts of the intestine where Crohn's usually

occurs. As he thought about chronic intestinal inflammation, Sartor said to himself, "It's the bacteria, stupid!"

He sought out the author of that article, UNC's own John Schwab, a microbiologist. "I told him I wanted to test his bacterial product to see if it could cause gut inflammation," Sartor says. "Most people thought I was out of my gourd. I had no research background. I was probably headed into private practice back home in Louisiana. But Dr. Schwab is a marvelous guy." He didn't think Sartor was crazy, and agreed to let him test his theory. And luckily Sartor had a little surgery background, so he was comfortable operating, even on a rat.

Working with Schwab, Sartor injected the bacterial product into a rat and waited. Within a month, the rat developed inflammation that looked a lot like Crohn's disease. It was a seminal finding that turned Sartor into a pioneer in a previously nonexistent field—normal intestinal bacteria implicated in inflammatory bowel disease (IBD). He never entered private practice, and he stayed at UNC, where he works with patients who struggle with gastrointestinal problems.

For years Sartor's team has bred germ-free rats and mice that scientists from around the world have used for research. In many experiments Sartor used mice deficient in the protective gene interleukin-10; these mice developed colitis, or inflammation of the large intestine. But IL-10 deficient, germ-free mice didn't get any inflammation. "Then, when we exposed those mice to typical gut bacteria, they got the disease within a week," he says.

UNC molecular biologist Ian Carroll, who works with Sartor, used the IL-10 knockout mice to see if a probiotic could help prevent inflammation. He gave mice *Lactobacillus*, a common beneficial bacterium thought to keep detrimental bacteria in check. The mice didn't experience as much inflammation. Then Carroll gave *Lactobacillus* to another set of mice, except this time the probiotic was engineered to release antioxidants in the digestive tract. The mice experienced even less inflammation.

The upshot is that certain strains of *Lactobacillus* might protect people from developing the worst kinds of inflammation associated with GI disorders. But that doesn't mean that probiotics help people if they already have Crohn's disease or ulcerative colitis, the causes of which are complex. Part of what's going on, Sartor says, is that Crohn's patients have genetic makeups that cause the innate immune system to react inadequately to the so-called bad gut bacteria. As a result, the adaptive immune system overcompensates—T-cells go on the attack against the normal intestinal bacteria and don't stop—causing inflammation that leads to symptoms such as abdominal pain, lethargy, diarrhea, fevers, weight loss, and bleeding. Most Crohn's patients are forced to have parts of their small intestines and colons removed. Eighty-five percent of the time patients require a second surgery within twenty years of their first operation.

Sartor had surgery in 1993 and hasn't needed another one. "I treat it traditionally with immunosuppressive agents, but I'm also compulsive about my diet and take an antibiotic directed against aggressive gut bacteria," he says. "I used to chug Coke and lots of sugary fruit juices. I don't do that anymore. I've pretty much cut out refined sugar from my diet."

Using the IL-10 mouse model, Sartor's team found that a high-sugar diet makes inflammation worse. "We also found that some bacteria groups grow in the presence of sucrose and fructose and others don't," he says. Sartor's group now uses the Microbiome Core Facility to profile these bacteria. The idea is to find out which species feed on sugar and cause bacterial imbalance in the gut.

To help limit symptoms and promote beneficial bacterial populations, Sartor eats prebiotics—fibrous foods that beneficial bacteria consume to grow their colonies. Leeks, garlic, onions, artichokes. If prebiotics help, what about probiotics? I asked Sartor if he took one. "I don't," he says. "There's no evidence that probiotics are effective for postoperative

Crohn's disease." One reason for that, he says, is that a lot of commercial probiotics don't contain the same species found in our guts. For instance, *Lactobacillus* and *Bifidobacteria* are genera that contain largely beneficial species. But the *Lactobacilli* and *Bifido* species found in probiotic pills and yogurt are not the same species found in us. Most don't colonize in our intestines, Sartor says, and don't necessarily alter the microbiota. What Crohn's and colitis patients might need

is a bacterial transplant, though the most current study results are not yet verified. In studies without control groups, Australian doctor Thomas Borody successfully treated colitis patients with human probiotic infusions—also known as fecal transplants. Without getting into details, the procedure involves repopulating the patient's colon with a healthier bacterial ecology (one found in a family member's fecal matter).

Brock Miller, a GI fellow at UNC, has used fecal transplants to help patients with *Clostridium difficile*—a nasty bacterial infection. In 2009 Miller had a patient who kept getting C. diff, so he recommended a fecal transplant. "She felt better the next day and hasn't had a relapse," Miller says. "A transplant almost always works for C. diff." Though the treatment is not yet approved for Crohn's or colitis patients, Sartor thinks IBD patients might benefit from altering their bacterial ecologies with a more specific approach using certain strains of bacteria.

Researchers in France, Sartor says, have found that Crohn's patients lack normal amounts of *F. prausnitzii*, a beneficial bacterium. Patients who relapse soon after surgery have especially low amounts of that bacterium. Sartor says, "In mice, those researchers found that *F. prausnitzii* protected against colitis."

Right now there's no *F. prausnitzii* pill at the health-food store, and doctors don't routinely prescribe other probiotics to IBD patients. But probiotics still might help the millions of people who scour drugstore aisles a little too often for relief from digestive problems.

THE "GOOD" BUGS

And why you want them

Probiotics date back to the early 1900s, when Russian scientist Elie Metchnikoff observed that Bulgarian peasants lived unusually long lives. He thought it was because they ate milk fermented by lactic acid bacteria. Metchnikoff started eating fermented sour milk made with a bacterium he called *Bulgarian Bacillus*, and reported that his health improved. His friends began eating it. Doctors in Paris, where Metchnikoff worked at the Pasteur Institute, began telling patients about it. After his death, Metchnikoff's bacterium was renamed *Lactobacillus delbrueckii subsp. bulgaricus*, which is one of the bacteria in some yogurts.

In 1920 scientists found that *L. d. bulgaricus* could not live in the human intestines; they doubted it had any benefit. The fermented milk fad faded in Paris, but research was just getting underway in the United States. In 1935 U.S. researchers surmised that *L. acidophilus*, a naturally occurring gut flora, would be more beneficial than *L. d. bulgaricus*. Clinical trials concluded that *L. acidophilus* helped relieve constipation. In 1989 probiotics were defined as live microbial feed supplements that beneficially affect the host animal by improving its intestinal microbial balance. Since then companies have cultured various bacterial strains and sold them as digestive aids.

"But not all probiotics are equal," Sartor says. Many of the probiotic products you can buy in stores have not been tested.





Tamar Ringel-Kulka, a UNC maternal and child health researcher, studied literature about the health benefits of probiotics marketed for kids and found that very few products had been investigated for effectiveness. Through a series of clinical studies, she and gastroenterologist Yehuda Ringel, her husband, have been investigating the beneficial effects of probiotics in children and adults. They're also trying to figure out how exactly these good bacteria work.

Ringel sees patients who have functional GI disorders for which there are no clear causes, such as inflammation or identified infections. Symptoms can include diarrhea, gas, bloating, nausea, constipation, and abdominal pain.

In one study, Ringel gave participants *Lactobacillus acidophilus* NCFM, a probiotic developed at NC State, and *Bifidobacterium Lactis* Bi-07. "The main effect was on bloating," Ringel says. "It was significantly reduced in patients who received probiotics compared to those who received placebos." The Mayo Clinic tested another blend of probiotics and also found that patients experienced significantly less bloating and gas. And, Ringel says, an RTP company got similar results after treating patients with an antibiotic that works only in the digestive tract.

Ringel and Ringel-Kulka are now studying the possible mechanisms by which probiotics relieve bloating. Previous animal studies revealed that L-NCFM can increase the activation of the colon's opioid receptors. "It may work like morphine," Ringel says. "If you activate the opioid receptors, you reduce sensation." You reduce the feeling of bloating. Ringel and Ringel-Kulka are now investigating whether something similar happens in humans.

Meanwhile, molecular biologist Ian Carroll is using UNC's Microbiome Core Facility to characterize bacteria taken from the people in Ringel and Ringel-Kulka's studies. The goal, Ringel says, is to gain a better understanding of the intestinal microbiota, especially in patients with functional GI disorders. Maybe a different probiotic would help more patients or alleviate different symptoms. Maybe a specific bacterial concoction can help prevent digestive problems.

Ringel-Kulka, a pediatrician, is doing that kind of work with children. She gave healthy kids aged one to four a yogurt drink containing *Streptococcus thermophilus*, *Lactobacillus bulgaricus*, *Bifidobacterium lactis* (BB-12), and the prebiotic inulin for sixteen weeks. After collecting biweekly journals that the children's parents completed, Ringel-Kulka found that kids who drank the concoction had fewer days of fever and significantly better quality of life, which in this context means that the kids felt better than usual and behaved better in day care than kids who were given a placebo.

"The changes we found aren't the major kinds of changes you see in patients who are ill," Ringel-Kulka says. "But that's why it's meaningful. The fact that you can improve health even further in healthy kids is pretty interesting."

That's especially true when you factor in that our symbiotic relationship with bacteria begins at birth.

THE BATTLE FOR BABY'S BELLY

Not all newborns have the same bacterial compositions.

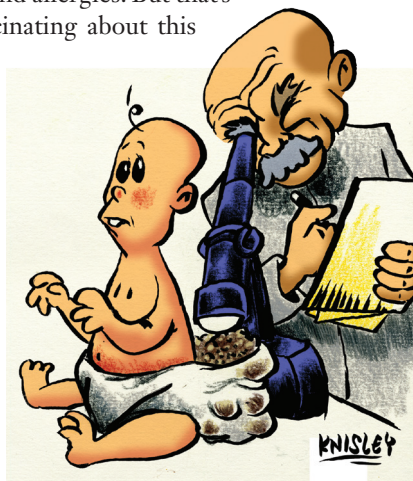
As a dad of two young kids, I was fairly confident that dirty diapers had no redeeming qualities. Apparently I've been misguided. These days, soiled nappies are good as gold to researchers interested in gut microbes.

Germ-free while in the womb, babies are exposed to beneficial bacteria, such as *Lactobacillus*, in the birth canal during delivery. Researchers are linking increased rates of childhood allergies and asthma to Caesarean section and the lack of a typical bacterial ecology at birth.

Breastfeeding also seems beneficial for bacterial balance. Sartor says that premature infants are susceptible to necrotizing enterocolitis, a devastating overgrowth of bad gut bacteria. "But that's almost universally found in formula-fed babies, not breastfed babies," Sartor says. Breast milk provides a steady influx of beneficial bacteria, he adds, "and there's abundant evidence that gut bacteria colonized early in life induce protective immune responses."

The theory is that vaginal birth and breastfeeding help babies acquire a healthy gut bacterial ecology, which helps the immune system ward off chronic ailments later in life. Right now findings are mostly correlative, not causative. But researchers, including some at UNC, have already found that diet affects babies' bacterial balances. The evidence is in the diapers.

As gastroenterologist Yehuda Ringel says, because we know so little about the human microbiota scientists can check how bacteria relate to just about any disease or disorder they can think of. And the correlations and causations between bacteria and disease are piling up.



BIG, BAD BACTERIA

Are gut microbes implicated in the worst kind of colon disease?

Five years ago I changed my diet and started taking probiotic pills to help with digestion. Within days I felt like a new man. But I never thought that a beneficial bacteria supplement might someday be able to help me avoid disease.

For most of his twenty-five-year career, Robert Sandler has been researching the causes of colorectal cancer, the second leading cause of death by cancer in the United States. “I was studying the same things everyone else was—diet, lifestyle, physical activity, family history, medication,” he says. “But the more I studied, the more obvious it was that there was something missing, because the data on diet are pretty inconsistent. Everyone thinks fiber protects against colon cancer. But if you look at the studies, some say it does and some say it doesn’t. About eight years ago I began wondering if gut bacteria play a role.”

Sandler says fiber might protect some people but not others against colon cancer because we all have different bacteria in us that react differently to fiber. He began working with Tope Keku, a UNC researcher who took the lead on clinical studies that try to determine whether bacteria have any say in whether someone gets colon cancer.

In 2010, Keku and Sandler used the Microbiome Core Facility to find that patients with precancerous growths or polyps in their colons have more Proteobacteria than people without the growths. Proteobacteria is a phylum that includes hundreds of species, including *E. coli* and other pathogenic bugs. Other researchers found that *E. coli* were more prevalent in biopsies taken from people with precancerous polyps and malignant tumors. And because bacteria fight each other for space in our bellies, the patients with more Proteobacteria and *E.coli* have fewer beneficial bacteria.

Still, Sandler and Keku can't yet say that bacteria caused the tumors. It could be that the tumors formed and then the bacterial balance was altered. "I think that's kind of far-fetched," Sandler says. "It's much more likely that bacteria play a role in the creation of the precancerous polyps."

Keku says, “*Something* upsets the balance of bacteria. It could be diet that leads to some bacteria growing faster than others. And then maybe those bacteria promote colon cancer.”

To find out, she's expanding her study to include hundreds of participants, and she'll use DNA sequencing to get a more precise view of which bacterial species are prevalent in patients with the kinds of polyps that often turn into cancer. She's also creating animal models to see if certain bacterial genera and species promote cancer growth. And if some do, then there ought to be a way to limit those so-called bad bacteria and promote the good kind to take up residence in our intestines.

It's not so crazy to think that someday those capsules full of bacteria and those creamy fermented snacks could include the sorts of good bacteria that are proven to prevent the fourth most common form of cancer in the United States.



“It would be great to find out what bacteria are missing and introduce them back into our diets,” Keku says. “What a great way to address colon cancer—you could know your risk and lower it by eating yogurt every day.”

BUGS IN THE LUNGS

More than a lone gunman

Matthew Wolfgang was fascinated by two simple truths: people with a specific genetic mutation will get cystic fibrosis (CF), and eventually, a nasty bacterium called *Pseudomonas aeruginosa* will dominate their lungs. Why *that* bacterium and not a different but equally bad bug? “We have no idea,” Wolfgang says. “We all encounter *Pseudomonas* every day. It’s abundant in soil and on plants. It likes to live in sink taps and shower heads. It doesn’t affect healthy people at all. But as soon as you become immune-compromised it becomes a problem.”

For years, CF researchers thought *Pseudomonas* was a lone gunman. But now they know that it has accomplices. Wolfgang used UNC’s Microbiome Core Facility to find dozens of bacterial species in the lungs of CF patients. “One is *Prevotella*,” Wolfgang says. “It normally resides in the mouth and doesn’t really do anything in healthy people. Occasionally it’s associated with abscesses. We’ve been finding *Prevotella* in high numbers in a lot of our CF patients.”

Wolfgang’s lab also found bacteria that can’t survive in oxygen-rich environments. “This tells you about where the disease has progressed in a patient,” he says. Some CF lungs are so damaged that oxygen can’t reach every part of the lung. Anaerobic bugs infect those areas, causing more inflammation.

Wolfgang says that the antibiotics CF patients take to kill aerobic bacteria are not as effective against anaerobic bacteria. “Maybe patients need combination therapies,” he says. “Maybe those *Prevotella* bacteria and others play some beneficial role.”

Wolfgang admits it seems crazy to think that *any* bacteria in the lungs would benefit anyone. Having no bacteria would be best. But a diverse community would be better than one dominated by pathogenic bugs. “It’s like in the gut,” Wolfgang says. “You want a balance. Bacteria fight for space, for nutrients.” You don’t want a really bad bug, such as *Pseudomonas*, to win that battle.

Wolfgang teamed with researchers in Ireland to study how bacterial communities change over time in CF patients. Turns out that as symptoms worsen and lung function decreases, the bacterial ecology of CF lungs gets less diverse. *Pseudomonas* becomes more dominant. The correlation is clear, but what to do about it isn’t.

CF patients typically take antibiotics that can have long-term side effects, such as kidney problems. Wolfgang points out that some bacteria in CF lungs are highly resistant to antibiotics. In the end, *Pseudomonas* dominates and causes major respiratory failure.

Still, trying to keep a CF patient’s lungs bacterially diverse might not be a good idea either. “I’m nowhere near advocating the use of probiotics—colonizing someone’s lungs with bacteria—because we know that the inflammation response to bacteria is doing a lot of damage,” Wolfgang says. “We’re getting a good handle on what’s going on in the lung, but we’re a ways off from figuring out how to intervene in a useful, therapeutic way.”

Balfour Sartor is a distinguished professor of medicine and microbiology and immunology in the School of Medicine, and director of the National Gnotobiotic Rodent Resource Center. Andrea Azcarate-Peril is an assistant professor of cell and molecular physiology and director of the Microbiome Core Facility. Ian Carroll is an assistant professor of medicine, Yehuda Ringel is an associate professor of medicine, and Brock Miller is a postdoc, all in the Division of Gastroenterology and Hepatology in the School of Medicine. Tamar Ringel-Kulka is a research assistant professor in the UNC Gillings School of Global Public Health. Amanda Thompson is an assistant professor of anthropology in the College of Arts and Sciences and a fellow at the Carolina Population Center. Kay Lund is the Sarah Graham Kenan Professor of Cell and Molecular Physiology. Tope Keku is an associate professor of medicine in the School of Medicine and an adjunct associate professor of nutrition in the UNC Gillings School of Global Public Health. Robert Sandler is a distinguished professor of medicine and epidemiology, and chief of the Division of Gastroenterology and Hepatology in the School of Medicine. Matthew Wolfgang is an associate professor of microbiology and immunology in the School of Medicine and a member of the Cystic Fibrosis/Pulmonary Research and Treatment Center.