The Ecosystem Inside

The trillions of microbes that live in the human gut could be the key to fighting disease without antibiotics.

By Michael Tennesen

IN THE INTENSIVE CARE NURSERY AT DUKE UNIVERSITY MEDICAL Center, doctors and nurses attend to premature infants in rows of incubators surrounded by ventilators and monitors. As new parents holding packages of breast milk watch their tiny babies, neonatologist Susan LaTuga makes her rounds, checking vital signs and evaluating how the infants tolerate feeding. She consults with nurses, dietitians, and pharmacists about the course of the day's treatment for the babies, some of whom weigh as little as one pound and were born as much as 17 weeks early.

At the end of her shift, LaTuga stops at a freezer and inspects stool samples from some of the infants that are at the center of a remarkable new study. Across the Duke campus, technicians are waiting to analyze them with a powerful gene sequencer capable of penetrating the hidden world of the billions of microorganisms growing inside each infant.

LaTuga is one of several medical researchers at Duke working

with microbial ecologists to study the development of the human microbiome—the enormous population of microbes, including bacteria, fungi, and viruses, that live in the human body, predominantly in the gut. There are 20 times as many of these microbes as there are cells in the body, up to 200 trillion in an adult, and each of us hosts at least 1,000 different species. Seen through the prism of the microbiome, a person is not so much an individual human body as a superorganism made up of diverse ecosystems, each teeming with microscopic creatures that are essential to our well-being. "Our hope is that if we can understand the normal microbial communities of healthy babies, then we can manipulate unhealthy ones," LaTuga says.

The Duke study is just one of many projects begun in the past five years that use genetic sequencing to explore how the diversity of the microbiome impacts our health. Two of the largest efforts are the Human Microbiome Project, funded by the National Institutes of Health (See "Your Microbial Menagerie," opposite), and the European Union's Metagenomics of the Human Intestinal Tract. Although these groups have only just begun to publish their findings, it is already clear that the microbiome is much more complex and very likely more critical to human health than anyone suspected. Understanding and controlling the diversity of our germs, as opposed to assaulting them with antibiotics, could be the key to a range of future medical treatments.

IN-DEPTH ANALYSIS OF THE HUMAN BODY'S MICROFLORA HAS BEEN possible only in the past few years—a by-product of the same new gene sequencing techniques that have allowed scientists to cheaply and accurately identify the DNA of the human genome. "Gene sequencing has opened a huge door to how complex these communities are," says Patrick Seed, a Duke pediatrician specializing in infectious disease, who with biologist Rob Jackson is a lead investigator of the premature infant study.

Before sequencing was available at a reasonable price, microbes were identified by growing them in a petri dish. But "not all microbes will grow in culture," LaTuga says. "It identifies only about 20 percent of the microbes in the gut."

Like a lush rain forest, a healthy microbiome in the human gut is a diverse ecosystem that thrives only when all the interdependent species are healthy too. "In an ecological sense, more diverse communities are healthy on land and in the seas," Jackson says. "No one species is dominant, and the ecosystem is more productive and resistant to major changes." The comparison is more than just a convenient analogy. Jackson was studying microbial communities around the world, including in the Amazon, when he realized that the ecological balance in those environments was not so different from the balance present in a healthy human gut. (One of his more counterintuitive findings is that microbial communities are more biodiverse in the American Plains than in the Amazon rain forest.)

Jackson's work on microbial diversity caught the attention of Seed, who was already interested in the microbiome in the guts of preterm infants but who did not have a background in ecology. He sought out Jackson, and the two decided to collaborate on what they call the Preemie Microbiome Project. The Duke medical researchers and ecologists who have joined that project hope to identify which species flourish in early stages of the human microbiome, how they are influenced by the consumption of breast milk, and what role they play in critical diseases affecting infants as well as in chronic diseases that occur later in life.

"The classical view of infectious disease is that a single organism invades and produces an infection," Seed says. "But then we found that certain diseases, like irritable bowel syndrome, seem to be caused by imbalances in the organisms that communicate with the host. So then people asked, 'Why is this not the case for many other states of human health?' " Preliminary work by other groups, similarly made up of both biomedical researchers and microbial ecologists, suggests that imbalances in the microbiome might also be linked to allergies, diabetes, and obesity.

The partnership between ecologists and biomedical researchers is characteristic of how things work in the relatively new but burgeoning field of microbiome studies. Vanja Klepac-Ceraj, a microbial ecologist by training and an assistant research investigator at the Forsyth Institute in Cambridge, Massachusetts, has helped organize symposia with ecologists and biomedical researchers giving joint talks on the ecology of disease. "Biomedical scientists understand disease, so they know where the problem lies within the body," she says. "Ecologists understand complex systems and the interaction of many organisms."

Klepac-Ceraj recently worked with Michigan State University ecologist Brian Maurer on a study of cystic fibrosis that showed the importance of microbial biodiversity in diseased lungs. Cystic fibrosis leads to mucus buildup in the lungs, which creates habitats for microbes and ultimately makes patients prone to lung infections. But their study of 45 cystic fibrosis patients showed that when the respiratory tract contains a more diverse community of microbes, the patient is less likely to harbor *Pseudomonas aeruginosa,* a key pathogen associated with later stages of cystic fibrosis. "The fuller and more diverse community correlated with a healthier outcome even though that community was not the model of a healthy lung," Maurer says.

MICROBIOME STUDIES RUN DIRECTLY AGAINST THE NOTION IN THE minds of most people—even many researchers—that microbes are linked to disease, not to health. And of course not all microorganisms are benign. Infants in particular are susceptible to a number of diseases caused by gastrointestinal bacteria, including sepsis, chronic diarrhea, and necrotizing enterocolitis, an infection of the intestinal lining that is one of the leading causes of

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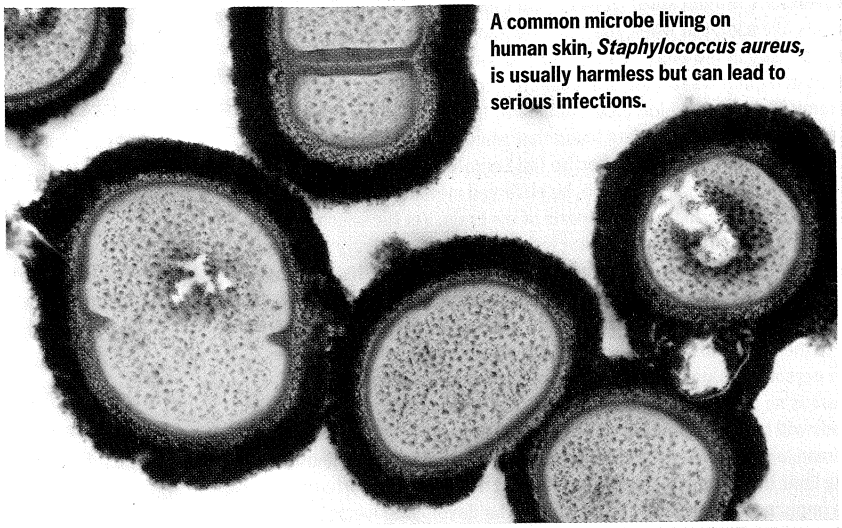
Your **Microbial** Menagerie

Two hundred trillion microscopic organisms–bacteria, viruses, and fungi–are swarming inside you right now. The largest collection, weighing as much as four pounds in total, clings to your gut, but your skin also hosts more than a million microbes per square centimeter. One population thrives among the hair follicles on your scalp, while an entirely different one resides in the crook of your elbow. About 1,000 species can live in the human mouth, where different sides of the same tooth sustain distinctly different combinations of bugs.

Surprisingly little is known about these invisible communities and how they affect us. In 2007 the National Institutes of Health (NIH) launched the Human Microbiome Project, a $115 million initiative exploring the bugs that exist in the human body, whether people all share a core population of such organisms, and how changes in microbial ecosystems influence human health and disease. In 2009 NIH geneticist Julie Segre published a study showing that physiologically comparable parts of the body host similar microbial ecologies, whereas contrasting areas–say sweaty underarms and dry forearms–have drastically different communities. "My scalp community is much more similar to your scalp than to my own back. That's because bacteria thrive in particular environments," Segre says. For instance, she notes, the face is ideal for *Propionibacterium acnes,* a bug that thrives on the oily, waxy remains of dead cells. "People often associate *P. acnes* with acne problems, but it also breaks down oils into a natural moisturizer for the skin."

The notion that the human body is teeming with hidden life may seem creepy, but our resident microbes seem to be overwhelmingly harmless. They educate the immune system and outcompete and block potential pathogens. For instance, *Staphylococcus epidermidis,* which lives all over the skin, prevents deadly staph strains from taking hold. "It's remarkable how Americans are so focused on sterilizing our exterior using antimicrobial products," Segre says. "Bugs throughout the body keep us healthy. We need to lose some of that language of warfare." AMY BARTH



A common microbe living on human skin, *Staphylococcus aureus,* is usually harmless but can lead to serious infections.

death in premature babies. Antibiotics have long been the first option in fighting these dangerous microbes, but many researchers are troubled by modern medicine's heavy reliance on them. After all, many pathogens found within the human microbiome are harmless or even beneficial. "There is *Staphylococcus* and *E. coli* in all of us, but they don't always cause problems," Jackson says. "It's the balance that is important. A more normal population of microbes in the gut can offset the bad players."

The Preemie Microbiome Project is an important step in understanding how we achieve a healthy, balanced microbiome in the first place. Researchers know that infants acquire about 100 species of microbes in the birth canal, and others come from the mother's skin after birth. As a child's contacts increase, some microbes are added from the doctor, the nurses, the proud dad, the doting relatives, and the curious family pets. By the time a baby is 6 months old, he or she has some 700 species of microflora, and by the end of the third year, each child has a microbial community as unique as a fingerprint.

Most of the infants enrolled in the Duke study are delivered by cesarean section, generally because the mother or the child has an infection or because the mother suffers from pregnancy-induced hypertension. Since they do not travel through the birth canal, "these infants come into life with virtually a clean slate, with few or no microbes at all," Seed says. "It gives us an opportunity to understand how the system works and develops."

The study also gives the researchers a chance to understand how antibiotics impact the formation of the microbiome. "Most premature infants are given antibiotics right away because of the dangers of disease," LaTuga says. "But more and more, we are learning antibiotics have multiple risks."

Heavy use of antibiotics can lead to antibiotic resistance, but researchers now speculate that antibiotics can also upset the balance of the microbial community, allowing disease to take over rather than fighting it. Michael Cotten, another neonatologist on the Duke project, analyzed the duration of antibiotic therapy given to 4,039 premature babies at 19 treatment centers across the country and found that prolonged use of the drugs is associated with increased risk of necrotizing enterocolitis and death. Antibiotics probably also prevent beneficial bacterial communities from forming in infants.

Last year, Stanford microbiologist David Relman published a study that illustrated the potentially devastating impact of antibiotics on the microbiome. He gave three healthy adults a five-day course of the antibiotic Cipro, then another course six months later, and monitored the state of the microbiome after each treatment. The gut flora of all three subjects gradually recovered from

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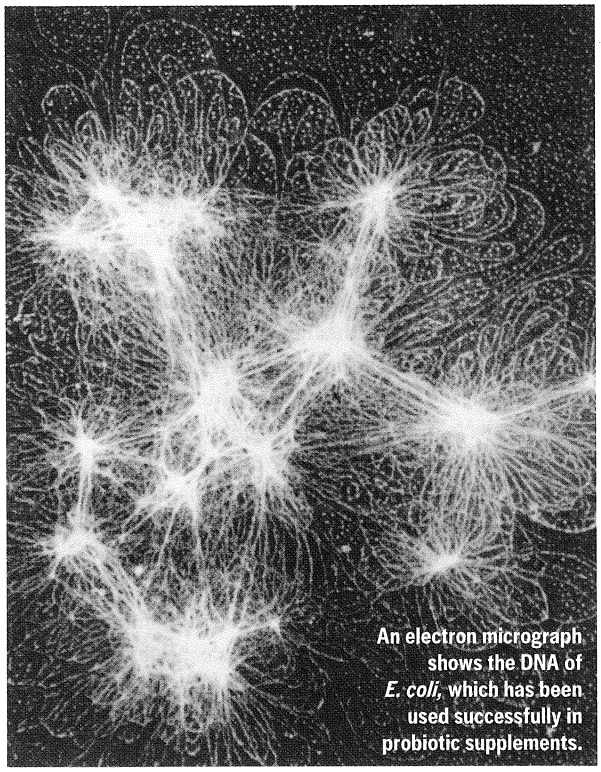
DR. KARI LOUNATMAA/PHOTO RESEARCHERS

Bugs **for** Breakfast

Around 9 million adults in the United States take "probiotic" supplements–pills packed with microorganisms such as *Lactobacillus acidophilus* and *Bifidobacterium lactis,* bacteria that are known to promote gut health. Foods containing microbial cultures, including yogurt, bear probiotic labels claiming they build immunity and improve digestion. Over the past five years, the U.S. probiotics business has grown almost 9 percent to about $5 billion a year, according to market research estimates.

But do probiotics actually improve the average person's health? Researchers are not sure. "A live yogurt with a few billion organisms sounds like a lot. But when you compare that with the trillions of organisms already in the body, it's a bit like throwing a packet of poppy seeds in a giant weed field and expecting to grow poppies," says Jeremy Nicholson, a biological chemist at Imperial College London. That said, Nicholson has found that some probiotics can have a dramatic impact. In 2008, when he fed *Lactobacillus* to mice with a transplanted human microbiome, he observed metabolic changes in the animals' gut, liver, kidneys, and parts of the brain. Yet Nicholson discovered that the animals' internal bacterial communities barely changed, suggesting that probiotics work by chemically signaling the microbes already living in the body, causing them to become more active.

Predicting the effect of probiotics on an individual is difficult. "A lot of them work in some people but not others because of differences in a person's biology, genetics, and environment," Nicholson says. There is no conclusive evidence that commercial probiotic pills and foods will benefit someone who is already in good health. But David Relman, a microbiologist at Stanford University, notes that malnutrition may limit the gut bacteria that help digest nutrients, exacerbating the impact of a poor diet. "Many kids in the developing world are not able to make efficient use of their food supply," he says. "A carefully constructed set of microbial strains could help them." **A. B.**



An electron micrograph shows the DNA of *E. coli,* which has been used successfully in probiotic supplements.

DR. ELENA KISELEVA/SCIENCE PHOTO LIBRARY

the impact of the antibiotic treatment but never returned to their original state—they had different compositions and were less diverse. "We don't know if these differences matter to health," Relman says. "But in general, you'd be concerned about a change." He had chosen Cipro because it has limited effectiveness against most species of bacteria in the gut, but it still affected one-third to one-half of the microbial flora in the subjects. "Knocking out one organism could have a ripple effect on the lives of others," Relman says.

This is especially concerning given that the number of different microbial species in the intestines may be important in countering pathogens. "The greater the diversity, the lower the probability that pathogens can invade and persist," says Richard Ostfeld, a disease ecologist at the Cary Institute of Ecosystem Studies in New York. "If all the niches are taken up in the gut, it might be hard for them to get hold."

Jackson puts it more bluntly. "When you use antibiotics, you are essentially dropping a bomb on a microbial community, hoping that your explosion will not harm anything useful," he says. "It's like setting a forest fire in order to control the weeds. What we're suggesting is to carefully manipulate the members of the community and the relationships between them, rather than wiping them out."

MANAGING THE MICROBIOME INSTEAD OF PUMMELING IT WITH antibiotics has produced impressive results in chicken and mice studies, pointing the way not just to future human treatments but also to a healthier food supply. For instance, increased use of antibiotics in chicken feed has led to an alarming growth of antibiotic-resistant bacteria in poultry. That resistance can get passed on to poultry consumers as well. In an effort to develop techniques to counter this worrisome trend, U.S. Department of Agriculture scientists introduced what they call a "competitive exclusion culture" of 29 different bacterial species into farm-raised chickens as part of their diet and then exposed them to salmonella. They found that chickens exposed to the bacterial culture had 99 percent less salmonella colonization than unexposed chickens.

In another animal microbiome experiment, Jeffrey Gordon, a biologist at Washington University in St. Louis, took a suite of microbes from the guts of both obese and lean mice and transplanted them into the guts of microbe-free mice. The mice that received the microbiomes of the obese mice gained significantly more weight than did the mice with the lean-mouse microbiomes. The results were the same regardless of whether the obesity of the donor mice was due to genetics or diet. Although caloric intake is still the most important factor in obesity, Gordon's research suggests that the microbiome may play a significant role by affecting the ability to extract energy from food and to deposit that energy as fat.

Researchers hope to achieve similarly dramatic results in humans next. A critical step in making this happen is deciphering how microbes communicate. "The establishment of healthy microbial communities almost certainly requires chemical messaging between the species present in the human host," says Texas A&M University biochemist Paul Straight, who studies interactions among bacteria. Microbes can use chemical signals, including small

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molecules, proteins, and DNA, to encourage neighboring organisms to grow or to tell them to stop growing. If researchers can capture and understand these molecular exchanges, they might be able to produce a kind of phrase book of chemical reactions. Such information could then be used to initiate this kind of molecular conversation on command, with an eye toward promoting the growth of helpful microbes or stunting harmful ones.

Specially packaged mixtures of microbes, known as probiotics, may also prove useful for balancing microbes in the gut (See "Bugs for Breakfast," opposite). Probiotics are now generally sold as health food supplements, and many of them are promoted as magic bullets that can improve metabolism or bolster immunity. Since they are as yet unregulated by the FDA, though, it is impossible for the consumer to know exactly what is inside; labels on over-the-counter products can be deceptive. Scientists who have tested them have often found something quite different from what the product promises. Nevertheless, carefully regulated probiotics, which introduce nonpathogenic competitors to disease, could be effective at balancing the gut microbiome.

THE RESEARCHERS ON THE PREEMIE MICROBIOME PROJECT ARE getting closer to understanding how we first acquire a healthy dose of inner microbes. They have finished sequencing the gut microbiomes of two groups of infants and have found that those infants are home to a surprising number of fungal species. Bacteria make up the vast majority of microbes in the adult body, so this finding suggests that fungi may play an unexpectedly important role both in the early development of microbial communities and in the health of infants.

Gene sequencing is also allowing Jackson and Seed to track down the sources of microbes, both benign and malignant, that find their way into the newborns in the study. They are uncovering evidence that mothers, breast milk, and hospital surfaces all contribute to the microbiome populations of infants. As part of this work, the researchers are linking pathogenic strains in the infants to specific instruments in hospital nurseries, information that could help doctors make the infamously contaminated hospital environment safer for vulnerable newborns.

The Duke team is also exploring whether the gut microbiome influences immune and metabolic development. If this turns out to be the case, it could be possible to introduce microbes that would improve infant immunity and metabolism. And the researchers are continuing to investigate the role breast milk plays in maintaining and encouraging the growth of a healthy gut microbiome. Breast milk is high in oligosaccharides, complex sugars that cannot be digested by the body but that may improve metabolism and immunity. By sampling the stool of premature infants and the stool and milk of their mothers, Seed and Jackson hope to understand how breast milk influences the timing of the appearance of different bacteria and fungi in the infant's guts.

Studies have shown that infants who are breast-fed are healthier, develop more quickly, and often have higher IQs. "We treat breast milk as a medication," says LaTuga, who believes that for now, breast milk is the best weapon doctors have to prevent infection in premature infants, reducing the need for long courses of antibiotics. "What is it that makes mother's milk so beneficial?" she asks. "How does it alter the gut microbiome to improve the healthy outcome of these babies? If we could answer those questions, we could help save infant lives."

The Duke group is still puzzling over how to translate their microbiome findings into practical treatments for premature infants. But at least one procedure that allows doctors to manipulate the gut microbiome is already here. A team led by University of Minnesota immunologist and gastroenterologist Alexander Khoruts has recently demonstrated spectacular success with fecal transplants, which introduce healthy stool microbes into a diseased bowel. An obscure and poorly understood procedure, it was first developed in the 1950s, well before anyone grasped the importance of the gut microbiome.

Khoruts and his colleagues reported last summer that they were able to use a fecal transplant to treat and apparently cure a woman with a life-threatening *Clostridium difficile* infection, which causes severe inflammation of the colon. The patient had an extremely poor prognosis: Suffering from chronic diarrhea, she had lost 60 pounds over eight months. "All antibiotics were failing, and she was in really bad shape," Khoruts says. In a last-ditch effort to improve her condition, he mixed a small sample of the patient's husband's stool with saline solution and injected it into her colon. Within 24 hours her diarrhea had stopped. After a few days, the symptoms were gone.

In studying this patient's progress, Khoruts was initially surprised to find that there was a nearly complete replacement of the woman's microbial flora with her husband's microbes. "By the time these patients get to this desperate treatment point, they've taken so many antibiotics that their microbiome has been decimated," he says. "So when we transplant the new bacteria, they simply move in to occupy the empty space." Before Khoruts and his team performed the procedure, no research had been done on how fecal transplants work or how they impact the microbiome. "Since then we've done another 23 patients," he reports, "all with dramatic stories."

As the cost of sequencing the human genome has plummeted in recent years, many medical researchers have touted the potential of personalized medicine—exotic therapies and synthetic drugs that are tailored to our individual genetic makeup. But "one day," Jackson says, "a genetic profile of our microbiome will be taken by doctors, with treatments prescribed from instant molecular data." The secret to keeping yourself healthy, it seems, might be to start by keeping your germs healthy.

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