THE ECOLOGY WITHIN

As an intern at the UPMC Eye & Ear Institute, Andrew Goldberg (Res ’90) met a middle-age man who swore he’d succeeded in curing himself of a pesky outer-ear infection after all his previous ear, nose, and throat docs had failed.

ENTs see this all the time: Give a patient a course of antibiotic drops, and maybe the problem clears up or maybe a chronic infection surfaces. So one day, this particular patient took matters into his own hands. Whatever’s in my good ear, I want to get over to my bad ear, he told Goldberg. So I just took some wax from my good ear and put it in my bad ear. And within a couple days, I was fine.
The story of the Earwax Man has become something of a legend among scientists in an emerging field, the study of what’s called the human microbiome. As Goldberg, professor of otolaryngology at the University of California, San Francisco, has shared this anecdote in his presentations, people have come up to him afterward sharing similar yarns dating back as far as the 1930s. One septuagenarian said his med school mentor swore by a cup of curative earwax that he kept in his office.

For centuries, improvements in hygiene and sanitation have saved innumerable lives. But as rates of asthma, allergies, and other inflammatory diseases steadily rise in the Western world, scientists now wonder whether we are seeing the cumulative effects of the pendulum swinging too far. It seems that, along with the bath water, we may have thrown out too many of the microorganisms that have co-evolved with us in a mutualistic relationship; we provided them a home, and they, in turn, keep our immune systems in check. With the rise of next-generation sequencing, which enables researchers to detect and sequence the DNA of all microorganisms within a given sample, we’re learning more about them than ever.

The balance of our bodily ecosystem is delicate, Goldberg explains. Think of it like a lake. If the temperature of the water increases, cold-water species decline. Algae become overgrown, choking off oxygen and killing helpful bacteria. Fish die, and the wetland birds that feed on them thin in number.

Many changes in our health have rippling and far-reaching effects we’ve never been able to explain; and increasingly, scientists are wondering whether the microbiome might hold the answers. For example, if you treat sinus disease, somehow, pulmonary disease improves, too. If a person develops lung disease, cardiovascular disease tends to go along with it. And study after study has contended that if a body takes probiotics down the hatch to introduce new microflora to the intestines—widely regarded as the throne of the microbiome, seating most of the microorganisms that populate our persons—a host of maladies ranging from asthma to psychiatric disorders quiet down.

The microbiome consists of trillions of microbes that make vitamins, ease inflammation, and influence everything from whether we can tolerate our breakfast cereal to how well our meds work. Their collective DNA has been called our “second genome,” and perhaps the best part about this genome is that, unlike the first one, which we're more or less stuck with for life, the microbiome’s is highly modifiable. When we give our microbial helpers what they need to seed and succeed, they spring back to health—and so do we.

A number of University of Pittsburgh faculty members are exploring this inner ecology, and University officials are in the beginning stages of planning a new microbiome center at Pitt (which is fertile ground for discovery in this area, with its strengths in interdisciplinary biomedical research, bioinformatics, computational modeling, and a wealth of clinical data available through UPMC, the largest academic health care center and payor/provider in the country). In October, Pitt awarded its 2014 Dickson Prize in Medicine (which honors a leading American investigator engaged in “innovative, paradigm-shifting biomedical research”) to microbiome luminary Jeffrey Gordon, who famously linked gut microbiota to both obesity in the Western world and undernutrition in developing countries.

In 2007, the National Institutes of Health (NIH) launched the Human Microbiome Project. Eight years later, industry is racing to cash in on its promise, and the public is clamoring for new hope in treating everything from autism spectrum disorder to food allergies. But in terms of evidence-based medicine coming to the clinic, we’re just not there yet, says Goldberg. On the upside, the idea of the microbiome is at least proving helpful in how he explains the disease process to patients—they understand the concept immediately and intuitively.

“Patients appreciate it, although I certainly wish I had something more concrete to deliver. It’s always, you know, It’s coming.”

**BRINGING UP BIOME**

One of the most troubling unsolved mysteries in pediatrics remains necrotizing enterocolitis (NEC). In severe cases of this disease seen in premature infants, sections of the intestines swell, wither, die, and must be removed, says Michael Morowitz, MD assistant professor of surgery at Pitt. About one third of NEC patients require surgery, and many of those children don’t survive after the procedure.

Since the 1990s, a number of likely contributing factors have been sleuthed out at Pitt, including inflammatory cytokines, nitric oxide, and a toll-like receptor called TLR4.

But all along, bacteria have remained a top suspect in NEC for several reasons: Many babies are cured of NEC with antibiotics. X-rays of these infants show guts full of gas bubbles—which are almost certainly produced by abnormal bacteria. And cases of NEC sometimes occur in clusters in a neonatal intensive care unit (NICU). Morowitz calls preemies the “Wild West” of the microbiome. Rather than spending their first days colonizing their bodies with typical neonatal microbes—from the birth canal, from their environments, and from milk—preemies are isolated in a NICU and take antibiotics intravenously. Those born before 35 weeks receive nutrition intravenously or through feeding tubes. “All bets are off,” he says.

In an NIH-funded study based at Magee-Womens Hospital of UPMC, Morowitz has been analyzing stool samples from preemies, prospecting this wild frontier. Thus far, NEC seems to be more about complex interactions—no rabble-rousing Lilliputian species (or gang thereof) has emerged as the culprit. So now, his team is
digging even deeper, developing techniques to sequence not just the DNA of microflora but also the RNA and proteins.

So far, Morowitz has learned that the preemie microbiome is guided by certain principles: First, preemies’ guts—regardless of whether they’re healthy or sick—are generally full of “very unfriendly” organisms. Second, even if two babies have the same bacterial species, each one will have her own individual strain that is markedly distinct, down to the level of individual genes. Third, the NICU environment is highly influential—preemies are populated by microbes found on the countertops, bed rails, and computers in the unit. And fourth, certain microorganisms with beneficial health properties in healthy newborns (e.g., *Bifidobacterium* and *Bacteroides*) are nowhere to be found in the intestines of premature infants.

Since his initial funding from the NIH, Morowitz has pursued other microbiome projects. In March 2014, he reported in the *Journal of Pediatric Surgery* a surprising difference he found between appendicitis patients and healthy youngsters. His team found *Fusobacterium* (best known as a gum-disease bug)—and lots of it—in the ill-fated appendixes, whereas control organs had none. This may help us better understand appendicitis, he says—which is another great unsolved mystery.

In another project, with collaborators Jillian Banfield, a PhD professor of Earth and planetary science and environmental science, policy, and management at UC Berkeley, and Joseph Carcillo, MD associate professor of critical care medicine at Pitt, Morowitz is studying the effects of the hospital environment on the gut, skin, and mouth microbiomes in children ages 1 to 9. (Most of what we know about the microbiome in this setting pertains to adults.) A few basic principles have emerged in these new Pitt studies, as well:

For one, the microbes of the skin and of stool samples—which should be very different—are actually quite similar in the ICU. “Which is not good,” Morowitz says. “My vision is that we’ll be checking these things twice a week, and if skin bacteria doesn’t look like it should, then [we’ll] do something about it. What that would be is up for debate.”

Typically, in a healthy body, microbes coexist: little populations of many, many stripes live together peaceably. “But what happens in the ICU you tend to get some individual, sort of obnoxious species that overtakes all others and grows to very high amounts.” (Other groups have shown in animal models that this can be a sign of an impending bloodstream infection, he notes.)

Also keeping NICU staff on their toes: In intensive care units, the microbiome changes drastically over the course of a few days. “Whereas in healthy individuals, it doesn’t change much over time unless they change their diet or start on antibiotics.”

Morowitz notes that clinicians generally don’t think much about bacteria, besides how to wipe them out in the case of infection. “But I think that over the next 5 to 10 years there will be a change, where all clinicians recognize that there are a lot of good organisms on the body, too, and part of caring for the patient will involve caring for the microbiome and making sure that the microbiome is appropriate for their age and place in life.”

**Gut Dealings**

In the early ’80s, Stephen O’Keefe, MD professor of medicine at Pitt, was investigating malnutrition prevention and treatment in rural Africa when he noticed something intriguing about his patients. As he was conducting lactose tolerance tests, which measured gases in the patients’ exhalations, he learned that much of the air rising from their guts to their gullets came in the form of methane—not hydrogen, as is typically the case in Westerners. Clearly, something very different was at work in what was then called the “bacterial flora.”

Years later, when he moved to the States and began practicing as a GI, O’Keefe was amazed to see how much more prevalent polyps and colon cancer were among African Americans than rural Africans—about a hundred times as prevalent. “And one of the obvious differences was their diet,” he recalls. The puzzle pieces began to slide into place.

With funding from the American Institute for Cancer Research, O’Keefe documented these dietary differences, then examined the
microbiota within the individuals and found a striking contrast between the two continents. In a 2013 paper in the American Journal of Clinical Nutrition, the team tested the hypothesis that it wasn’t so much the food itself that determined colon cancer risk, but rather, how the food affected the microbiota, and in turn, the byproducts—or metabolites—that they produce.

A little gut primer: Your small intestine lives on the same goodies you live on, the amino acids, fats, and sugars forged by your digestive system. But by the time your food reaches the end of the line—the colon—anything absorbable is gone. What’s left is indigestible residue—fiber, that is—which microbes in the colon process and break down into short-chain fatty acids. Among them is butyrate, which is not only the major energy source for the cells within your colon, but also their peacekeeper. Butyrate controls the rate of cell turnover and proliferation in the organ’s lining. It also suppresses inflammation and keeps the walls snug and secure, protecting actively dividing cells inside from troublemakers, like carcinogens.

Essentially, butyrate is your ultimate colon cancer queller—a gift of your gut bacteria, for the small price of room and board.

The 2013 paper showed the correlation between metabolite production and colon cancer risk. In April, Nature Communications published an NIH-funded follow-up study showing this mechanism at work in the two populations whose striking contrast first gave O’Keefe pause 30 years ago: rural Africans, with their high-fiber and low-protein diet, and African Americans, whose diet is exactly the opposite. O’Keefe and his Dutch collaborators, Willem de Vos and Erwin Zoetendal of Wageningen University and Research Centre, showed that by switching the diets of these populations for as little as two weeks, their butyrate levels flip-flopped.

And so did their rates of epithelial-cell proliferation—a biomarker for colon cancer risk.

**FUNGUS O’LUNG-US**

When the NIH announced the Human Microbiome Project, they invited grant proposals for studies of the nose, mouth, skin, gut, and urogenital systems, but not of the lung. Even as recently as 2007, the thinking was that the lung—though incessantly exposed to the external environment—was sterile. You see, when you culture lung tissue samples, you almost never get anything to grow, unless that person has a raging infection like pneumonia, explains Alison Morris (MS ’03), MD associate professor of medicine and immunology at Pitt.

The NIH—specifically, the National Heart, Lung, and Blood Institute—soon came around, and Morris and Elodie Ghedin, a PhD and former Pitt faculty member who is now a professor of biology and public health at NYU, applied for and were awarded a spot in the multicenter Lung HIV Microbiome Project.

COPD (chronic obstructive pulmonary disease) is on the rise in people with HIV. Morris’s was among the first groups to investigate the reasons why. When starting out as a scientist, she studied a fungus called *Pneumocystis jirovecii*, a common cause of fatal pneumonia in the early days of the AIDS epidemic. She went on to show that it plays a part in HIV patients’ continued vulnerability to COPD, as well.

In comparing the lung microbiomes of HIV-positive and HIV-negative people, she has found surprisingly similar bacterial populations. However, there are some significant differences. Her studies with the Lung HIV Microbiome Project show that the lungs of people with the virus are far more likely to house *Tropheryma whippelii*, a bug that causes a rare disease in the GI tract. (It does not cause the GI disease in these HIV patients. “We don’t yet know how it gets [in the lungs] or what it’s doing,” says Morris.)

In addition, people who are on antiretroviral therapy for HIV actually have worse airway obstruction than HIV patients who are not. “What we think may be going on is that as their immune system gets better, they may be more able to react to things”—including the harmless flora of the microbiome. “That [reaction] causes inflammation that damages the lung.”

Getting in on the ground floor of the lung microbiome took a lot of front-end work; lung tissue sampling requires an invasive procedure called bronchoscopy. Luckily, the Pitt Men’s Study—a longitudinal public health research initiative that’s been following HIV-positive men in Pittsburgh since the start of the epidemic—has provided a wealth of samples and dedicated volunteers.

Yet, bronchoscopy accesses the lungs through the oral and nasal route, which Morris calls a “veritable sewer.” Microbes along the respiratory tract move, mix, and mingle as we breathe and cough. Establishing how best to minimize the nonlung microbes in the samples, and rule out the ones that do manage to sneak in, took three years. Computational modelers from the University of Michigan helped sort out which microorganisms thrive in which environment.

With lessons learned from the Lung HIV Microbiome Project, Morris and collaborators launched a new study called GRADS (Genomic Research in A1AT and Sarcoidosis). Its foci, sarcoidosis and alpha-1 antitrypsin deficiency, are two very different diseases, but both are suspected to be at least partly fueled by infection. GRADS will integrate for the first time information from our first and second (microbiome) genomes as well as clinical information on these diseases in the hope of identifying biomarkers that can help physicians track disease progression and response to therapy. Co-led byNaftali Kaminski (formerly of Pitt and now MD professor of medicine at Yale) and Steve Wisniewski (PhD professor of epidemiology at Pitt Public Health), GRADS is funded by an $8.3 million grant from the NIH. Now nearing the end of its highly successful enrollment stage, the multicenter study is shaping up to be the largest study of both of these diseases to date.

**IRRITABLE BIOME**

In any ecosystem, from the rivers of Allegheny County to the vegetable garden in your backyard, the health of the community goes to pot when a species gets too greedy and crowds out its competition. The same goes for the microcosm in your gut. In one extreme and dreaded example of this, a complication of antibiotic therapy known as *Clostridium difficile* infection, the little bacterial dictators can bring you to death’s door. And sometimes, the cure—ironically, more antibiotics—can do you in.

Hence, the growing acceptance of a new therapy called fecal microbiota transplantation (FMT), which is exactly what it sounds like: transplanting fecal specimens from healthy donors to reseed a doomed intestinal tract with diverse microflora (under close medical supervi-
sion, of course). It may soon become the standard of care for *C. diff*—trials at medical centers around the world, including UPMC, have seen high success rates thus far.

Some scientists are hopeful that FMT might help sufferers of other diseases that are also believed to result at least partly from imbalances in the intestinal microbiome—namely, ulcerative colitis and Crohn’s disease, which together are known as inflammatory bowel disease, or IBD. Michael Morowitz (see “Bringing up Biome”) and Alka Goyal, MD assistant professor of pediatrics at Pitt, are conducting a novel FMT trial for IBD in pediatric patients at Children’s Hospital of Pittsburgh of UPMC.

To hear Pitt’s David Binion, MD professor of medicine, tell it, there’s no more perfect place to study the role of the microbiome in IBD than here—quite a vote of confidence given that he himself has Crohn’s. Pitt, he points out, is the home of Richard Duer, MD professor of medicine, of human genetics, and of clinical and translational science, who for the last 15 years has been a major player in characterizing IBD’s implicated genes, particularly those involved with the interrelationship between the immune system and the microorganisms in the GI tract.

Pitt, Binion says, has three coauthors of the American College of Gastroenterology’s *C. diff* physician guidelines: Binion himself; Scott Curry, MD assistant professor of medicine; and Brian Zuckerbraun, chief of trauma surgery and an MD associate professor.

Pitt is in collaboration with MIT on a project called OpenBiome, which is screening FMT donors and banking the samples so that patients in desperate need can get FMTs more quickly.

“And I’ll go ahead and say it,” Binion adds. “We are one of the leaders when it comes to handling observational natural history data”—that is, systematically tracking every imaginable metric of the patients who’ve consented to participate (that adds up to some 16,000 clinical visits, 2.4 million lab results, and 112,000 prescriptions so far) and linking them to clinical outcomes. This unique asset has been lacking in gut microbiota research until now, he says.

“You can generate all the information in the world about genetics and the microbiome, but then you have to link it to the human data. That’s where we believe the discoveries are going to come from.”

**UPWIND AND DOWNSTREAM**

Goldberg, of the famous Earwax Man story, has actually spent his entire microbiome-research career investigating microorganisms not in the ears but in the sinuses.

Several years ago, he became involved with a study of biofilms—the sticky, antibiotic-resistant microbeasts long suspected as the reason why some sinus infections tend to spring back no matter how many different antibiotics you throw at them. The effort evolved into an intensive, years-long project that in 2012 resulted in a game-changing *Science Translational Medicine* paper. Therein, Goldberg and his UCSF collaborators proved once and for all that the sinus cavities, in both sickness and in health, are loaded with microflora; for centuries, we’d assumed the sinuses were sterile, except in disease states. The difference, the team found, was that healthy people had more diversity of species, more even distribution among them, and greater bacterial counts overall.

And for the first time ever, their paper showed that something akin to a probiotic for the sinuses—introduction of helpful bacteria to chronically inflamed cavities—could prevent infection in an animal model.

Goldberg continues to learn more about the teeming community within your face; several papers are out for review. In one, his team defines subcategories representing the range of courses chronic sinus infection can take—four “sinotypes” that are each microbiologically and immunologically distinct. In another paper, he traces what he believes to be the mechanisms behind the link between improved sinus health and improvement in asthma symptoms, a curious downstream effect that has long confounded explanation.

Eventually, Goldberg hopes to go back to where this all started for him, the ear. But as yet there aren’t enough resources to do so.

“We have a bandwidth problem,” he says. “There are so many things we want to do.”

But, you know. It’s coming.