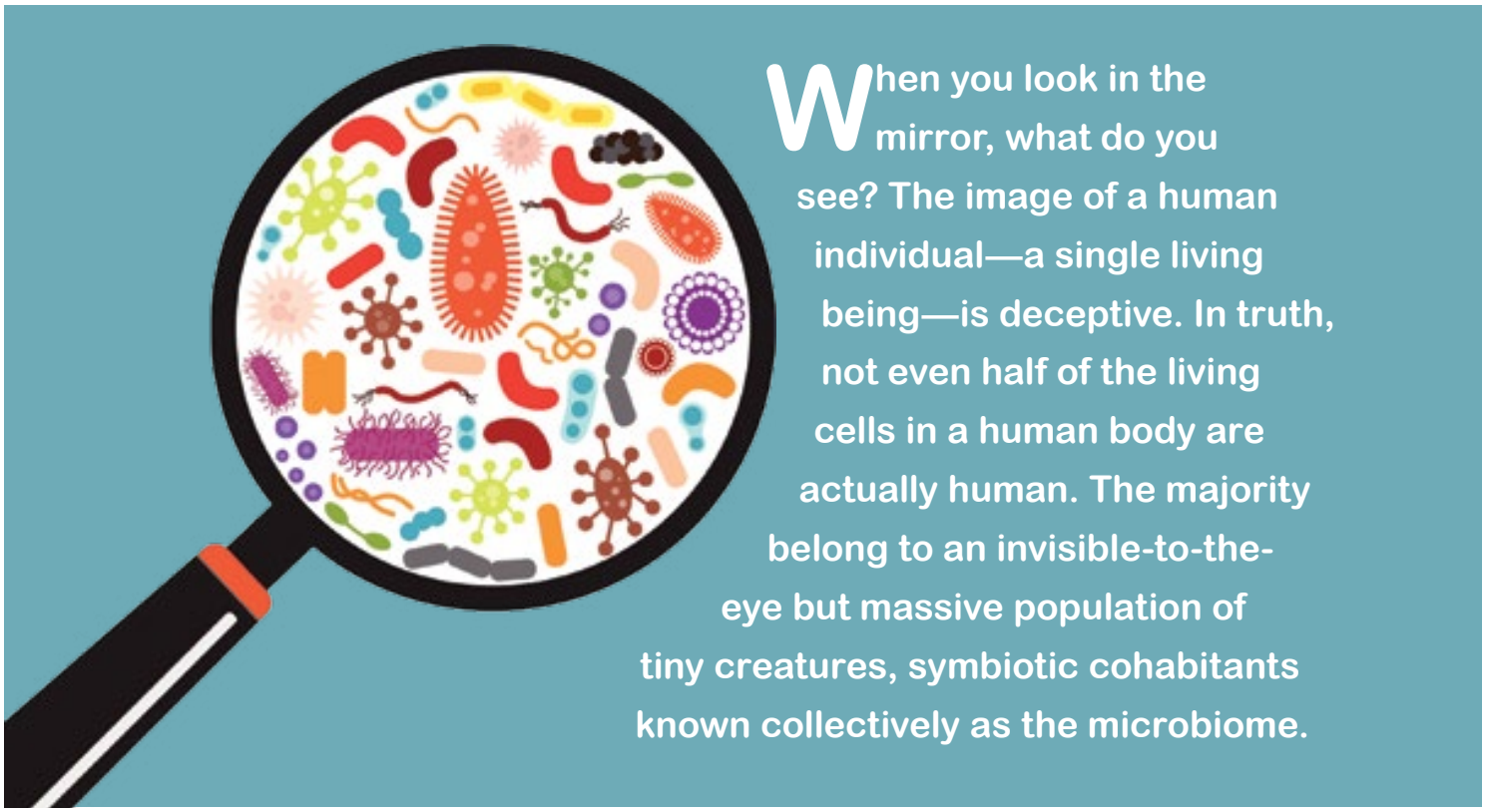
By Claudia S. Copeland, PhD

THE WORLD WITHIN US:

Health and the Human Microbiome



When you look in the mirror, what do you see? The image of a human individual—a single living being—is deceptive. In truth, not even half of the living cells in a human body are actually human. The majority belong to an invisible-to-the-eye but massive population of tiny creatures, symbiotic cohabitants known collectively as the microbiome.

THE MICROBIOME is made up mainly of bacteria, but also includes fungi, protists, and even archaea (nonbacterial prokaryotes often found in extreme environments). This microbial population is extremely diverse, and in fact consists of several different and mostly independent communities, each adapted to its own environment. The microbial community in the intestinal tract is very different from that in the vagina, and these are both very different from that in the mouth. Microbial populations live all over our body, from our lungs and gut to inside our nose, mammary glands, seminal fluid, ovarian follicles, and of course all over our skin. Even the insides of our eyelids serve as habitats for microbes.

So, in a sense, we are mini-environments for large communities of tiny life forms. We provide these microbes with nutrients, water, and protection from dangers like the UV radiation in sunlight. But what do they provide to us? Are they just silent residents taking advantage of all our bodies have to offer them, and giving back nothing in return? Or is it a two-way street–do they also exert actions on us, their living environment, that affect us in subtle or serious ways?

Until recently, that question was virtually impossible to answer in any sort of comprehensive way. In the past, research in natural microbial populations in both internal and external environments was difficult to conduct. Traditionally, in order to study a microorganism, you would need to culture it. In order to culture a bacterium (or other microbe), you need to create a culture environment in which it can thrive, which requires understanding the biology of the microbe, plus a lot of time and effort in trying different culture conditions before getting one that works.

Since natural microbial communities are composed of countless species, most of which have not even been named, let alone studied to the point of being able to culture them, it was simply impractical to study these microbial communities as a whole. Instead, most microbiology focused on microbes of disease significance–those bacteria, viruses, and parasites that are NOT normally found in the body, and that cause illness when they are. The "good microbes" quietly living their lives in and on our bodies, were ignored.

Then, in the 1980s, molecular biologists started to realize that you could study populations of microbes in environmental samples in essentially the same way individual genomes were starting to be studied–on the basis of their DNA sequences. In 1988, a team of University of Indiana researchers led by Dr. Norman Pace began a study using PCR to directly amplify highly conserved parts of the 16S ribosomal RNA gene from Pacific seawater samples collected off the coast of Hawaii. Since all bacteria use 16S ribosomal RNA, and parts of the gene encoding this RNA are extremely conserved, amplifying this gene in a sample can "catch" virtually all of the bacteria in that sample, even completely unknown species. In this way, microbiologists could not only study bacteria they did not know how to culture,

but they could also study bacteria that they didn't even know existed–species that were completely uncharacterized–and they could study all of the bacteria in a sample, as a whole community, at once.

In 1991, this team, Schmidt et al., published their analysis of a marine picoplankton community, characterized as a whole on the basis of DNA sequence data. Since then, laboratory techniques have become increasingly sophisticated, and in 2004, J Craig Venter and colleagues published the first pilot whole-metagenome analysis of marine microbial samples from the Sargasso Sea. That is, building on the techniques they developed to sequence the human genome, they were able to sequence the entire "genome" of a sea sample. On the basis of this multi-organism community genome, called a metagenome, they found over 1,800 species, including 148 previously unknown bacterial phylotypes. With over a billion base pairs of whole-genome sequence data, they were able to elucidate not only species but also gene content, diversity, and relative abundance of the organisms within the sampled community. In addition to identifying new species of bacteria, the whole-metagenome approach allowed them to identify over a million previously unknown genes.

At this point, the field of metagenomics was a fully birthed baby, with methods and technology that could be developed for use in any type of metagenomic study. Today, in addition to large-scale environmental studies such as the Earth Microbiome Project, researchers are now conducting a number of human microbiome studies, including prominent large-scale studies like the NIH Human Microbiome Project, the Metagenomics of the Human Intestinal Tract (MetaHIT) consortium, a joint project of the UK's Sanger Institute and the European Commission, and the American Gut Project, an ongoing crowdsourced project directed by biologists Rob Knight and Jeff Leach. It is from these large-scale projects, as well as a number of smaller academic studies, that we have started to scratch the surface of what the microbes living in our bodies do for us.

The Human Microbiome and Human Health

It turns out, these microbes do quite a lot. For a start, beneficial bacteria can help protect the gut from pathogens via a competitive exclusion effect. Simply put, the "good bacteria" crowd out the "bad bacteria" that would otherwise cause disease. But they do much more than that. A healthy gut microbiome is protective against allergies, obesity, type 2 diabetes, irritable bowel syndrome, Crohn's disease, and ulcerative colitis (Inflammatory Bowel Disease), and even mental health conditions like depression.

Among the most surprising associations is a link between the microbiome and Parkinson's Disease. The etiology of Parkinson's Disease has long eluded biologists. It does not have a strong genetic component, and is not an infectious disease. Epidemiologically, the pattern of the disease suggests an environmental cause, and several toxins have been implicated, but none have been found to exert a strong enough effect to be considered responsible. Several studies have observed that the gut microbiome in Parkinson's patients differs markedly from that in healthy people. Beyond association, however, are preliminary results that indicate causation, including mechanistic studies in mice that point to neuroinflammation resulting from microbial matabolites, and a transplantation study, in which University of Wisconsin researchers Sampson et al. transplanted fecal material from human Parkinson's Disease patients and normal, healthy human controls into genetically susceptible mice. The mice that received the fecal transplant from healthy donors continued to be healthy, but the mice that received the Parkinson's patients' microbiome began to show Parkinson's-like symptoms.

While data linking the microbiome to Parkinson's Disease are new and sparse, the microbiome link for other disease areas has been clearly shown. When gut microbiomes from lean or obese donor mice were introduced into microbe-free mice, the mice who received the obese microbiome became obese, while those who received the lean microbiome stayed lean. Further, germ-free mice given the microbiomes of obese humans or their lean twins had the same effect on their phenotype–the microbiomes from obese humans made the mice obese, while the microbiomes from lean humans did not. This was the case even

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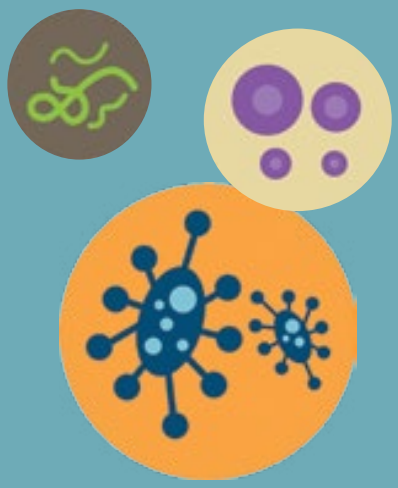

though germ-free mice are relatively resistant to weight gain, compared with conventional mice. Insulin resistance has also been reversed in mice upon transfer of a healthy microbiome.

In humans, microbiome transfer via fecal transplant has been astonishingly effective for patients infected with *Clostridium difficile*, a bacterium that causes severe diarrhea. Patients who had suffered for years from *C. difficile* found their symptoms disappearing mere days after fecal transplant from a healthy donor. Today, fecal transplant is a standard therapy for *C. difficile,* with 90% success rates in patients who had been unresponsive to antibiotic therapy. Fecal transplant is also being studied in irritable bowel syndrome, Crohn's Disease, and Ulcerative Colitis patients. Clinical trials are also underway for microbiome transfer as a treatment for obesity, metabolic syndrome, and non-alcoholic fatty liver disease. Donors should be chosen carefully; a case report of a *C. difficile* patient who received a fecal transplant from an obese relative reported that the patient, who had never been obese, experienced an increased appetite and rapid, unintentional weight gain. To stay on the safe side, therefore, most guidelines now specify that fecal donors should not be obese.

One of the most intriguing and mysterious areas of microbiome research is the effect of the microbiome on mental health and behavior. Pennington Biomedical Research Center biologists Annadora Bruce-Keller and colleagues compared mice who had received fecal transplants from donors who had been fed a high-fat diet with those receiving transplants from donors fed a normal diet. The mice receiving the high-fat microbiome exhibited significant disruptions in exploratory, cognitive, and stereotypical behavior. Recently, they have extended their research to look at the behavior of offspring of mice with different microbiomes. They transferred two sets of microbiomes, one from lean mice fed a healthy diet and the other from overweight, metabolically unhealthy mice fed a high-fat diet, into pregnant mice, and looked at the consequences for the babies. They found that the pups of mothers given the unhealthy microbiome had a number of behavioral differences from the pups of mothers given a healthy microbiome. Differences continued to be seen into adulthood. Male offspring in particular were affected, with the sons of unhealthy-microbiome mothers showing significantly increased behaviors associated with anxiety and cognitive impairment.

Probiotics vs. Prebiotics

While fecal transplant is a promising treatment for some serious disease conditions, most non-diseased people seeking to cultivate a healthy microbiome probably do not want to go that far. Instead, if "good bacteria" are good for our health, can we just take probiotics to get those good bacteria into our bodies? The short answer is, no, for a number of reasons, according to Dr. Frank Greenway of the Pennington Biomedical Research Center. First, it appears that a healthy microbiome is a diverse microbiome; there is no one bacterial species or small group of species that are "the good ones." Not only do probiotic pills only contain a few different species of bacteria, but many of the bacteria in a healthy, diverse microbiome are anaerobic and too fragile to survive being pressed into a pill, swallowed, and immersed in a hostile, low pH environment, says Dr. Greenway. In addition, if you do manage to introduce some good healthy additions to your microbial population, but then eat a narrow range of low-fiber processed foods, these microbes will essentially starve to death.

Instead, Dr. Greenway says, the focus needs to be on feeding the microbiome to encourage a good mix of microbes. Towards that end, he and his colleagues have been investigating the clinical efficacy of various prebiotics in fighting chronic diseases like diabetes. While he emphasizes that a diverse mix of unprocessed food is a good way to maintain wellness, for those already suffering from a chronic illness like diabetes, a more proactive approach may be needed.

"It seems that, when people have a chronic disease like, say, diabetes, they tend to have certain dysfunctions in terms of a normal

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microbiome," Dr. Greenway explains. "And, so, one of the ways that one could approach that is to try and find things that the bacteria would like to eat, that would encourage the 'good guys' to restore the normal functions of the biome." He believes that dietary supplementation can help grow beneficial microbes that help to restore these normal functions.

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While each component of this multicomponent GIMM, called NM504, has benefits in its own right, NM504 is designed to be administered in combination with metformin, a traditional first-line diabetes treatment. The results in diabetic patients have not only been positive, but in some cases, dramatic. Dr. Greenway describes the case of a patient whose fasting glucose had only dropped from 375 mg/dl to 325 mg/dl with metformin treatment, but who then decided to supplement with NM504. "About 20% of the people who take Metformin develop GI problems with it, and he was one of those people. After about nine days on Metformin, he was just having constant diarrhea," recalls Dr. Greenway, "Well, after two days on the supplement, his blood sugar had dropped to about 175, and his diarrhea was completely resolved." After just 8 weeks of taking NM504 along with the metformin, the patient's fasting glucose had dropped to 100 mg/dl. In addition, the watery diarrhea he had experienced as a side effect of the metformin disappeared when the treatment was supplemented with NM504. Further, at one point, the patient had run out of the supplement, and his diarrhea returned. After a few days, when he was able to begin taking the supplement again, his diarrhea once again resolved.

"We were pretty impressed by that, and metformin does make changes in the microbiome, so we did another study that we published to take people with intolerance to metformin and had them take either a placebo or supplement for two weeks and showed that, in groups of people, that it reduced the side effects of metformin. That's the sort of thing we've been doing– trying to look at the microbiome from the perspective of its function more than just looking at the individual bacteria that are in it."

The results of the pilot trial, published in the *Journal of Diabetes and Its Complications* in 2015, found a clear improvement in glucose tolerance in patients with fasting blood glucose levels between 100 and 200 mg/dL (pre-diabetic or diabetic) who had taken NM504 and metformin compared with the group who took metformin only. In addition, the NM504 group experienced increased satiety compared with the control group. Dr. Mark Heiman, chief scientific officer at MicroBiome Therapeutics, emphasizes that metformin is a safe and effective drug, and that "NM504 is not a substitute for metformin, but it may be used with metformin to help individuals better tolerate the GI side effects and thus help them continue to obtain the benefits of taking the medicine as prescribed by their doctors."

MicroBiome Therapeutics is also developing a product for obesity, called MT303. It is made from "activated" soybean pods,

which contain phytoalexins, chemicals that are released in plants when they are stressed. "If you stress the young soybean pods by cutting them," explains Dr. Greenway, "they make these phytoalexins, which in the case of soy is called glyceollin." Dr. Greenway describes how mice fed MT303 ate more than control mice, yet lost weight. Clues to this paradox were found in the stool: the stool samples contained increased amounts of triglycerides, indicating that, rather than being absorbed, much of the fat these mice were ingesting was just passing through their systems. "When they fed these activated pods to rodents who were on a high-fat diet, it turned out that there was an increase in the stool volume. There wasn't any diarrhea, just more stool."

Dr. Heiman is looking towards what MT303 can do for humans. Beyond just helping people lose weight, he explains that MT303 "has been shown in mice to impede the absorption of fat into the blood from a high fat diet. This may be useful in helping to maintain healthy fat levels in the liver and blood in individuals with a number of disorders such as nonalcoholic steatohepatitis."

In addition to diabetes and obesity, Dr. Heiman is looking towards the myriad of other health conditions affected by the microbiome. Compared with new drug development, GIMM development can move forward much faster, since these are natural dietary supplements–technically, they are food, not drugs, and there are no side effects beyond those of eating foods like soybeans and blueberries. While Dr. Greenway stresses the importance of clinical trials–after all, there are thousands of natural products that purport to treat any number of conditions, but which do not do so effectively, or, necessarily, safely–he also told of how soybean pods, from which MT303 is derived, used to be a regular part of the diet in the Midwest in the 19th Century, and were eaten in China in ancient times. It is ironic that after centuries of progress and development, our diets have actually become impoverished in ways we are just now beginning to understand.

Dr. Heiman hopes to put the emerging knowledge about the microbiome and the prebiotics required for it to flourish to good use in improving human health. He takes a holistic view of how understanding the microbiome can improve our health. "We and others are discovering and developing GIMMs from specific isolated micronutrients in foods to offer a safe means to deliver key nutrients, which may be deficient in a diet and are necessary for a healthy functioning GI microbiome. The microbiome within our gut responds to nutrients we eat, the drugs we take orally, and substances we ingest to supplement our diets. It is too early to define all responses of the GI microbiome to each of the entities we consume but it is very likely that our choices determine the composition and function of our GI microbiome. Scientific investigations are beginning to define particular signals produced by the GI microbiome that communicate with our physiological systems. These associations are revealing novel connections for health, but there are also suggestions that some products from the microbiome may participate in disease states." His enthusiasm for the promise of this novel area of biology is palpable: "It is like discovering a new organ within us that is primarily modulated by our dietary choices." ■

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