CENTERING ON THE GUT

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We've all taken and taught courses that cover the digestive system. After a while it gets to be old hat: stomach acid, peristalsis, intestinal villi, and so on. That's why I was so surprised, when going through my stack of articles, to find several that deal with the gut in very novel ways. It seems that the digestive system is hot right now and at the nexus of a number of other current topics in biology from genomics to immunology, from ecology to microbiology. Surprises like these make me want to keep teaching biology because they mean that I can never teach it the same way twice. There's always something new to enliven things.

○ Genomics

As with many other areas of biology, digestive-system research is getting a makeover because of genomics. Obviously, knowing more about the human genome has given researchers information on the genetics of gut-related diseases like colitis and Crohn's disease, but it is the study of microbiological genomes that I refer to here. Metagenomics, the investigation of a host of genomes at once, has opened up to examination the organisms living in and on the human body. Many of these are bacteria, the majority of which cannot be cultured with standard microbiological techniques. This means that they have been essentially invisible to researchers. But with the advanced genomic analyses available now, whole bacterial communities can be studied at once. This has led to a much greater appreciation for the diversity of organisms that we carry around with us, our internal ecosystems.

Researchers estimate that there are 10 times more microbial cells than human cells in the body (Cerutti, 2010) and that these organisms belong to many species. Almost 130 species have been identified in the stomach, more than half of which were previously unknown. This is in the *stomach*, which in the past was thought to be almost sterile because of its great acidity. That was before another biological surprise: that peptic ulcers could be caused by an infection with the bacterium *Helicobacter pylori*. Needless to say, the intestine possesses an even richer microbial flora. There are over a thousand different bacterial species in the human gut, and there are large-scale projects in progress right now to sequence at least portions of all their genomes (Zhao, 2010).

By large-scale, I mean multinational and long-term (Mullard, 2008). There are several of these projects, and there are still questions as to how, eventually, they might relate to each other. The United States is working on a Human Microbiome project dealing with microbes all over the body. The aim is to create a "reference set" of genetic sequences for 900 bacteria which can serve as a source of information for future genetic and metabolic research. In an article on the results so far, the Human Microbiome Jumpstart Reference Strains Consortium (2010) reported that 356 genomes have been generated, including the upgrading of 117 genomes, meaning that the data are being checked and gaps filled. Of the other 239, there are 151 from the intestinal tract, 33 from the urogenital/vaginal tract, 28 from the mouth, 18 from skin, and 8 from the respiratory tract. There is also a single isolate from the blood. The dominance of the intestinal tract here is obvious.

The European Commission is homing in on the gut with its Metagenomics of the Human Intestinal Tract project (MetaHIT). Although there is some coordination between the projects in the United States and Europe, they are essentially working independently. The massive size of each is one reason for this autonomy. Just organizing communication within each project is a formidable challenge, and there are other projects to take into consideration as well. MetaHIT is working together with a Chinese project called Meta-GUT, and there's also the French MicroObes project and the Human Gastric Microbiome in Singapore. Just to add to the mix, the Australians are working on the urogenital microbiome, and the Japanese also have a human-metagenome consortium. As sequencing becomes cheaper and easier to do, data will continue to proliferate. But what does it all mean, and who will figure out this meaning?

One result of the availability of all this data is something called "pan-genomes" – multiple sequences for the same species, in most cases for bacteria. A pan-genome is the sum of both the "core" genes shared by all the sequences and the "dispensable" genes that are found in only some of them. For example, *Lactobacillus reuteri* has a core genome of 1600 genes. There are seven different *L. reuteri* sequences available, and with this largesse it's becoming obvious that the bulk of genes in this bacterium have been identified, few new genes being added with each new sequence. This is important information because it means that after a certain point, researchers can, in a sense, close the book on a species and go on to use their resources for sequencing other bacteria.

○ Ulcers & Viromes

Other kinds of interesting studies are being done on particular gut bacteria – for example, on how to control the bacteria involved in such conditions as diverticulitis. A German group has tracked the geographic spread of the ulcer-causing *H. pylori* (Linz et al., 2007). Work in the late 1990s indicated that strains of the bacterium differed genetically in Asia and Europe – as, of course, do human populations – but it required the development of new statistical tools to discover whether or not this was more than a coincidence. The research team found variations among *H. pylori* strains on different continents that were consistent with its movement with human migrations for at least the last few thousand years. This work, which has the feel of an unfolding mystery story, then led into population genetics, with analysis of more *H. pylori* strains

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from populations in the Basque region of Europe, central and southern Asia, and Africa. Finally, the bacterium could be tracked statistically all the way back to the time about 58,000 years ago when anatomically modern humans migrated out of Africa. With all the dogged analysis done here, what does this study really show? First, that *H. pylori* is hardly a recent arrival in the human gut. It also gives us one more piece of information about our ancestors, as well as verifying in a new way when the move out of Africa occurred.

Along with all this work on bacterial genes, there are also studies of viromes, the genomes of viruses and virus-like particles. Again, one of the prime areas under investigation is the gut, and fecal materials are good sampling sources (Reyes et al., 2010). In one study, researchers monitored the feces of pairs of adult female monozygotic twins and their mothers over the course of a year. In contrast to the bacterial genome, which is more similar among twins and their mothers than among unrelated individuals, the virome was equally variable in both groups. There was great diversity between viromes of different individuals, regardless of their degree of relatedness, but little variation over time in the virome of a particular person. Many of the viruses identified were phages, so the bacterial genomes and the viromes are related to each other, a can of worms that at this point is in most cases too complex to attempt to open. However, it suggests that our personal ecosystems have still another layer of complexity hidden within them.

○ Focus on Feces

The subject of feces is not often raised in polite conversation. However, biologically speaking, they are a wonderful source of research specimens, and they provide a window into the very closed world of the gut. Those of us who have endured colonoscopies and/or endoscopies know just how difficult it is to investigate that world closely guarded by sphincters. When looked at from this perspective, feces may not seem quite so foul. One rather interesting area of fecal investigation stems from a little-known type of transplantation: that of the digestive system.

An article by Apoorva Mandavilli (2008) describes the case of a young woman who had such a transplant after suffering through years of gradual destruction of her digestive system by familial adenomatous polyposis, the growth of thousands of benign tumors in the digestive tract. Finally, its last remnants were removed, and she received a healthy digestive tube, from esophagus to anus. Not surprisingly, such a transplant isn't always successful, and it has to be carefully monitored for indications of function on one hand and rejection on the other. Fecal samples are taken regularly, and as Mandavilli notes: "The end of the gut that would normally go into the rectum is left poking out of the abdomen so that doctors can check the transplant is stable" (p. 581). This stoma makes biopsy easier, and it's also a good way to get fecal samples and study how bacteria go about recreating ecosystems in the gut. The very presence of feces is obviously a good sign, and these feces are checked periodically for their changing microbial riches.

This brings up a crucial aspect of transplanting the digestive tract. Because this tube is literally full of bacteria and surgeons are extremely careful to create an aseptic environment for their work, it has been standard protocol to use antibiotics to rid as many organisms as possible from the "new" digestive tube. Now, researchers who study the development of bacterial ecosystems after transplantation are questioning this approach. They've found that the earliest settlers in the intestines are enterobacteriaceae, which are facultative anaerobes, which means that they can grow with or without oxygen. These organisms can indicate the presence of infection, since inflammation can increase oxygen levels. In the normal gut, there's little oxygen and most microbes are anaerobic. Also, after transplantation there are often rather wide shifts in the makeup of the bacterial population, whereas large fluctuations don't usually occur in the microbial profile of a healthy gut. The more chaotic the ecosystem, the less likely that the transplant will be successful. These data suggest that severely disrupting the flora with antibiotics may not be the best approach in gut transplantation, especially given that when more antibiotics were used, there were more subsequent infections.

This is one of those cases where experimental results seem to go against "common sense," and it takes what amounts to a leap of faith to ignore common sense. Transplanting a "dirty" gut just doesn't seem right, but so far, the results of taking this tack have been encouraging. Transplants are more likely to be successful with this approach, and the intestine moves toward normal functioning more rapidly. Obviously, careful monitoring continues to be necessary, but this is a good example of how sometimes it's better to let the body do its own work than to force human intelligence upon it. With this new method, facultative anaerobes are still more common soon after transplantation, but a complex and more anaerobic population develops more quickly.

○ Disease & the Gut

With the information now available from metagenomic projects, researchers have also discovered a great deal about how microbial communities function under less severe conditions than those of transplantation. One expected finding is that there are variations in microbial communities among individuals. What is also becoming clear is that these variations may be related to a number of human diseases. Right now, this is an exciting and fast-developing area of research, but one that is based on relatively scant information compared with what there is to know both about the gut itself and about what lives there and why. Reading articles on this work, I got the sense that the excitement at finding this rich world may be outpacing the amount of hard evidence available to understand how it operates. This is not necessarily a bad thing. It is at such times, when enthusiasm acts as a spur to experimentation, that great progress can be made. However, these are also times during which it's best to be cautious about accepting new results. Gut microbes are being linked to everything from obesity to colitis, from diabetes to heart disease. All these connections may turn out to be valid, but if we introduce our students to this wonderful new world, we should also present some caveats as well: research in the middle of being done is exciting, but at the same time it is, by its very nature, tentative.

Researchers come up with these correlations between microbial populations and human health primarily by comparing the fecal ecosystems found in different individuals (Pennisi, 2009). As mentioned earlier, metagenomics has shown that the microbial flora differs from person to person, but also over time and even within parts of the intestine. When feces of infants are analyzed over the course of the first year of life, a progression in bacterial species is seen as microorganisms come to populate the gut. The organisms themselves change the environment there, to say nothing of how the flora changes with diet over that first year. Comparisons show that the flora of bottle-fed babies is different from that of breast-fed infants, and babies delivered by Caesarean section have different microbes from those delivered vaginally. The latter have the "advantage" of picking up microbes

from the mothers' vaginal and anal areas. Identical twins have more similar flora than other siblings, but these bacterial communities are hardly identical, which suggests that gut ecosystems are so rich in species interactions that these never occur in quite the same way in any two individuals.

This diversity continues throughout life, and even increases, as these ecosystems respond to differences in diet and to other metabolic fluctuations. For example, obese individuals tend to have different gut floras than those without excess weight, and when the former lose weight, their bacterial populations can change and become more like those of the non-obese (Pennisi, 2009). Further investigation of this phenomenon in mice has revealed the involvement of the immune system. It makes sense that immune cells would be particularly important in the gut, with its large bacterial population and its constant exposure to new organisms introduced in food. One type of immune cell produces what are called "toll-like receptors," which recognize bacterial flagella and trigger a response (Sandoval & Seeley, 2010). Mice that lack toll-like receptor 5, which is expressed by intestinal cells, tend to be obese, and when these animals are given antibiotics, the obesity is reversed. In addition, when mice with this mutation and with depleted gut microfloras are exposed to bacteria from the obese mice, they too become obese. In this instance, obesity looks like an infection. It could be that particular bacteria are able to extract calorie-rich molecules from food that ordinarily doesn't get digested, and this excess nutrition leads to the accumulation of excess fat. Other researchers suggest a different hypothesis: that the gut bacteria don't provide extra calories, but rather influence metabolic processes that control the accumulation of fat.

Before getting too excited about these results, remember that this research was done on one mouse strain under controlled conditions. However, it definitely suggests ties among the bacterial ecosystem in the gut, the immune system, diet, and body weight. It's worth further investigation, which, of course, it is getting. So are a number of other studies linking these elements, and dealing with parts of the immune system aside from the toll-like receptors. For example, immunoglobulin A (IgA) functions to impede harmless bacteria in the gut from entering other parts of the body (Cerutti, 2010). The dangers of peritonitis indicate how important such safeguards are, and how pernicious such "harmless" bacteria can become when let loose. IgA is a weak antibody, just what's needed in this situation to keep the bacteria at bay, yet not remove them from the gut, where they make vitamins, aid digestion, and keep infectious organisms in check. If, however, bacteria do breach the defenses, IgG is produced, which recruits phagocytes to counter the microbes. So, in the gut as elsewhere in the body, there's a complex interplay of immune responses to balance vigilance against overreaction.

This interplay may be an important factor in a phenomenon that has become much more prevalent over the past few decades, "metabolic syndrome." This term refers to a cluster of problems ranging from obesity to insulin resistance, from high levels of blood lipid to high blood pressure (Li & Hotamisligil, 2010). The syndrome involves both genetic and environmental factors, one of the latter being diet, and it therefore has an association with the digestive system. I've already mentioned the link between intestinal bacteria and obesity. Besides toll-like receptors and IgA, the immune system has another protein called NOD1, which can be activated by short-chain fatty acids generated by microbial activity in the gut. Each of these immune components is usually studied separately, and it appears as if what researchers are doing is creating islands of knowledge in a sea of ignorance, or perhaps it is better to refer to three intermingling seas: immunity, digestion, and microbes.

I've put my toe into each of those areas here, but I feel that I haven't examined even a thimbleful of any of them. I can't see going into detail about any of them in class, but I think it's good for students to at least be aware of such complexity. In light of this, I want to mention another study that brings up yet a different realm: that of intestinal macrofauna or parasites and how they, too, are involved with bacteria (Hayes et al., 2010). The parasitic nematode *Trichuris muris* infects the mouse's large intestine. Its hatching depends on the presence of enterobacteria such as *Escherichia coli* and *Salmonella typhimurium*. Hatching was triggered by the bacteria's fimbriae, which bind to proteins at the poles of the worm's eggs. Also, when the mice were treated with antibiotics, the number of nematodes decreased markedly even though these parasites aren't directly affected by the antibiotics. Here is just another small piece of a huge puzzle, and one that may be intriguing enough to share with our students because it involves so many topics we routinely cover in general biology.

○ Beyond the Gut

While I've obviously been gut-focused here, I'd like to note that microbial diversity is also true of the skin, the vagina, the nasal passages, and other nooks and crannies of the body. Let's face it, some bugs must even find ear wax tasty. Over the years, when describing the microbial life we carry around with us, I've told students that when they leave class they should appreciate that several ecosystems are leaving with them. I didn't realize until recently how truthful I was being. A number of observers of the biological scene have commented recently on this diversity and forced me to think more broadly about its significance. The biologist Robert Dorit (2008) argues that "it's time for a new conservation-minded view of the microbial communities that live on and in us" (p. 284). By this he means that as we come to appreciate just how complex these communities are, we should also come to realize that reconstructing them would be difficult. In other words, broad-spectrum antibiotics that destroy not only the intended target but a whole swath of microbial life could be the equivalent of indiscriminant aerial bombing – perhaps effective at wiping out a target, but not worth the price because of the devastating collateral damage.

Dorit makes the point that genomic studies not only provide information on the variety of microbes that call us home, but also, as the full genomes of pathogenic organisms are worked out, reveal how they cause trouble in the body. In other words, researchers are identifying the specific molecules that interact with our cells and lead to damage. In this way, it's becoming more likely that specific remedies can be found to interfere with this interaction and short-circuit the microbe's attack. Then, perhaps, only the target bug and not "good" ones can be removed from the body's ecosystem, allowing the beneficial species to remain. While keeping in mind that just as pinpoint bombing is often less than perfect, no drug is without side effects, this approach seems worth pursuing.

As Dorit suggests, this tactic involves more than just a different strategy for pharmaceutical companies, it also means developing a different mindset among doctors and patients. Microbical ecosystems deserve more respect! This is one reason I'm writing this column, and if you want to begin heightening your own awareness of this issue, Dorit's article is a good place to begin. He does a great job of describing how these ecosystems develop and how it comes about that "the skin on our right forearm, for example, harbors a different microbial community than that of our left forearm…. Even single regions of the body, such as the vagina, consist of multiple defined subenvironments, each with its own subset of tenants" (p. 286).

David Barash (2008) is a psychologist who often writes on human evolutionary issues. In his discussion of our many ecosystems, he takes a broader view and discusses not only microbes but eukaryotic residents as well. He notes that parasites can manipulate host behavior, so we may not be as autonomous as we suppose. The example he uses is from the murine world, but it does involve the gut. Barash describes how "the tapeworm *Echinococcus multilocularis* causes its mouse host to become obese and sluggish, making it easy pickings for predators, notably foxes, which – not coincidentally – constitute the next phase in the tapeworm's life cycle" (p. B18). He also looks at the other end of the spectrum, the viruses that we carry around in our chromosomes. Endogenous retroviruses take up more than four times more space in the human genome than all the coding genes. Barash uses these statistics to question precisely what being human means, with all these vestiges of viruses past carried with us while at the same time being grossly outnumbered by prokaryotes and a smattering of other critters as well. Pretty soon there'll be a genome hunt for our fungi, which I'm sure will also yield surprising results.

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