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DIARRHEA DIGEST

DIARRHEA DIGEST is an irregular publication of TECHLAB®, Inc. dedicated to the etiology, diagnosis, and therapy of diarrheal diseases and related aspects of intestinal ecology

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Powerful S#!t --- Stuff

We all know that feces can have a powerful odor, but did you know that it can be used to generate power? Approximately 50% of the energy available in an animal's diet is passed out in the fecal matter, therefore a lot of energy remains to be harvested by organisms such as decomposers, or exploited by engineers and scientists as a source of renewable energy.

In 1894, Englishman Joseph E. Webb patented the "sewer gas destructor lamp" to handle the gases that developed in the sewer systems as a byproduct of the natural decomposition of human waste by microorganisms through a process called anaerobic digestion. Biogas (sewer gas), which contain a lot of methane, were collected in domed structures built into the sewer system, then piped to area street lamps where they were mixed with the town gas supply and combusted. This was done primarily as a means of disposing of the combustible methane to prevent it from building up in the sewer system, not as a source of renewable energy.

In the early 20th century, many of these lamps were erected in towns across England, such as Sheffield, which at one time had more than 80 such lamps. One of the most famous of the sewer gas lamps is located on Carting Lane in London, giving rise to the nickname of "Farting" Lane. Modern sewage treatment facilities continue the practice of burning off the methane produced in the sludge to dispose of it, but on a much larger scale. When I was a child, my family frequently traveled to visit my grandparents in Richmond, Virginia. We usually left on Friday night, and near the Richmond city limits, there was a sewage treatment plant. High above the sewage treatment plant burned an eternal flame, a glowing testament to the energy available in feces.

There are approximately 16,000 sewage treatment plants in the United States, 1,000 of which process a large enough volume of waste in which it is economically feasible to recover the energy available in the sludge. Currently, most of these plants simply burn off the gases, wasting the energy. If the energy available in the sludge was harvested at only half of those plants and used to generate electricity, an estimated 340,000 homes could be powered (1). In effect, what was flushed down the toilet would come right back into the home through the power lines in the form of electricity.

Again, we travel to the United Kingdom, where GENeco, a company headquartered in Bristol, operates 5 hybrid sewage treatment/power plants throughout western England. This gas is burned on-site and produces more than enough heat and electricity to run the plants. The excess electricity is fed into the national power grid and provides enough electricity to power 10,000 homes each year. At similar facilities located at several correctional facilities in Rwanda, biogas generated on-site from the waste of the incarcerated provides half of the energy needed by the prisons (2).

If further refined to pure methane, biogas can be used to power vehicles as well. The Bio-Bug, a Volkswagen Beetle retrofitted by Greenfuel Company for GENeco, is powered by methane gas generated and refined at GENeco's Bristol sewage treatment plant. It is unlikely that we will all be driving "dung beetles" one day, as it takes the annual waste from 70 homes to power the Bio-Bug for a year; therefore biogas alone is not a replacement for gasoline, but it could make a dent in the amount of fossil fuel needed to power the world's vehicles - Sweden currently

has over 10,000 vehicles powered by biogas. While the idea of using natural gas (methane) to power vehicles is not new (probably a late 1800s idea), using human waste as a source of that gas is fairly new.

Another clever use of sewage to generate electricity has been implemented at Thames Water in London, England. In addition to burning the biogas, the sludge is dried and cut into "poo cakes" that are burned to produce additional electricity. The poo cakes are similar in nature to charcoal briquettes but, disturbingly, look a lot like brownies (3).

Still in the developmental phase, but promising nonetheless, are microbial fuel cells that can produce electricity from sewage without the need of a combustion step. Bacteria naturally produce protons and electrons as they metabolize the nutrients available in the sewage. In a microbial fuel cell, the flow of these electrons and protons between a cathode and anode is exploited in a type of biological battery (4). Researchers at the University of Colorado Denver used a small microbial fuel cell powered by wastewater to power the lights on a Christmas tree (5). Researchers at both the J. Craig Venter Institute in San Diego and at Penn State University have also successfully generated electricity using microbial fuel cells (6, 7). Improvements in fuel cell technology, combined with genetic modifications to the microbes, could significantly improve the efficiencies of the prototype fuel cells, making them a reality. Electricity from microbial fuel cells, combined with the energy generated by burning biogas and poo cakes, could one day provide a significant portion of the electricity needed by the industrialized world.

In non-industrialized nations, however, there are generally neither sanitary sewer systems nor other hygienic means of disposing of human waste, the lack of which fosters the spread of communicable disease. To help combat this problem, the Bill and Melinda Gates Foundation has provided $44 million in research grants to "spark new innovations in sanitation" (8). Some of the projects funded by these grants are small-scale, self-contained combination toilet/sewage processing plants utilizing some of the same technology discussed above, but with the primary objective of processing and decontaminating the waste rather than generating electricity.

A team at Loughborough University in England is developing a toilet that uses heat to convert human waste into charcoal, salt, and potable water through a process called "hydrothermal carbonization". The toilet would be self-powered, as all of the energy required for processing the waste would be generated by burning the fecal charcoal (8).
Another toilet, currently under development by a Dutch group at Delft University, would blast the waste with microwaves. The resulting syngas, a mixture of hydrogen and other gases, can be used to power a fuel cell to generate electricity, which would in turn power the microwave, plus provide a small amount of additional electricity that could be used for other purposes. A similar fuel cell approach is being investigated at the California Institute of Technology, but instead of a microwave, a small reactor powered by solar panels on the roof of the unit decomposes the waste, producing syngas as a byproduct, which in turn powers a fuel cell (8).

The above toilets may sound like science fiction, but they could one day improve the lives of the billions of people currently living in areas without a basic sanitation infrastructure.

On a lighter note, a dog park in Cambridge, Massachusetts, uses methane obtained from dog waste, deposited by responsible dog owners into a tank system called the "Park Spark" to power a gas lamp that lights the park at night. While not saving a tremendous amount of electricity, it does inform the public of the potential power of poop. Employees at the Denver Zoo modified a motorized rickshaw from Thailand, called a tuk tuk, to run on pellets composed of dried animal dung and waste generated by park visitors. When heated, the pellets produce syngas, which is burned to power a generator

which produces electricity to power the tuk tuk's electric motor. Since more waste is produced at the zoo than is needed to power the tuk tuk, zoo engineers are considering a larger version of the syngas system developed for the rickshaw that could provide 20% of the zoo's electrical needs while simultaneously diverting 1.5 million pounds of waste from local landfills, which will provide an additional $150,000 in savings annually.

Another, more peculiar, syngas-fueled vehicle is the Toilet Bike Neo, an unusual contraption devised by Japanese toilet manufacturer TOTO. The driver's seat is a toilet (not a functioning one), but contrary to what some sites have reported, the "turd trike" is not a "poop-n-go" vehicle powered directly by the driver's waste. This odd contraption has a talking miniature toilet on the handlebars, plays music, and can even write messages in the air as it rides by using a technology called residual light imagery. Through the efforts of forward-thinking engineers and scientists, the above technologies could one day provide a portion of the electricity needed to power the world, with the added benefits of reducing greenhouse emissions and demand for fossil fuels. Syngas, biogas, fecal charcoal, and microbial fuel cells: all proof that feces truly is powerful stuff.

Jeremy Boone

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Norovirus --- #1 in diarrhea

Norovirus, formally know as Norwalk virus, is the most commonly detected pathogen in fecal samples from patients suffering from acute gastroenteritis. Twenty-three million cases of gastroenteritis are caused by norovirus each year, which contributes to about 70,000 hospitalizations and 800 deaths. Norovirus accounts for >90% of all *viral* gastroenteritis outbreaks and about 50% of *all* gastroenteritis outbreaks [1]. Twenty-six percent of the fecal samples from patients visiting emergency rooms for severe diarrhea are positive for norovirus

Norovirus was first discovered in human fecal samples from a gastroenteritis outbreak in Norwalk, Ohio in 1972, hence the name Norwalk virus. Several viruses associated with diarrhea have been identified since then. Viruses from four families cause diarrhea: *Reoviridae* (rotavirus), *Caliciviridae* (norovirus and sapovirus), *Astroviridae* (astrovirus) and *Adenoviridae* (adenovirus). Noroviruses can be divided into 5 genogroups (GI, GII, GIII, GIV and GV). There are 8 genotypes in GI, 17 in GII, 2 in GIII, 1 in GIV and 1 in GV. Genogroups GI, GII and GIV are found in humans, while genogroups GIII and GV are found in cattle and mice. A subset of GII genotypes are also found in swine. In recent studies, Genotype GII.4 was implicated as the cause of 70% of norovirus outbreaks worldwide.

Noroviruses are relatively simple. The virions are envelope free; the external capsid measures 27 to 30 nm in diameter. Noroviruses are single-stranded positive sense RNA viruses. The RNA genome of norovirus is approximately 7.6 kilobases in

length and encodes 3 open reading frames (ORFs), which encode 8 proteins. The first ORF encodes 6 proteins, including the RNA-dependent RNA polymerase. The second ORF encodes a capsid protein, and the third ORF encodes a protein that interacts with the virus' genomic RNA during virion formation. The third ORF is the most variable region in the genomes of various genotypes of norovirus [2]. Murine norovirus contains an additional ORF4, which overlaps with ORF2.

Norovirus is mainly transmitted via the fecal-oral route. Viruses can become airborne in case of explosive vomiting. Norovirus outbreaks have been reported in hospitals, schools, cruise ships, hotels and restaurants. Several outbreaks were associated with contaminated vegetables and oysters. Noroviruses are extremely infectious. The infecting dose is less than 20 virus particles. The secretion level is 10^8 to 10^{10} copies of viral RNA per gram of feces and virion secretion can continue for more than 2 weeks after symptoms resolve. One report indicated that norovirus RNA was detected in an individual the day before the onset of symptoms.

Clinical symptoms of norovirus infection include nausea, abdominal pain, vomiting and diarrhea. The incubation period from exposure to symptoms is 24 to 48 hours and the symptoms last 12 to 60 hours. Around 30% of norovirus infections are asymptomatic. Asymptomatic carriers can be sources of community-acquired infection, although transmission of norovirus in hospitals is mainly caused by symptomatic patients. The current CDC guideline states that people who experienced symptoms of norovirus should not return to work until 2-3 days after clinical recovery. Norovirus can survive for 28 days at 20°C (room temperature) and 60 days at 4°C (refrigerator temperature). Alcohol-based hand sanitizers that inactivate viruses that depend on a lipid envelope are not effective against norovirus because norovirus does not have a lipid envelope. Careful hand washing with water and soap is more effective than alcohol-based sanitizers. Noroviruses can be inactivated by heating or by chlorine-based disinfectants. Surfaces in hospitals can be decontaminated with 2% hypochlorite.

Upon entering the digestive track, noroviruses can survive the acid in the stomach and infect and replicate in the cytoplasm of the enterocytes that line the intestine. The putative cell-surface receptors for norovirus are histo-blood group antigens (HBGAs), which are blood group carbohydrates expressed on intestinal epithelial cells. Different norovirus strains bind to different HBGA carbohydrates. Hosts which lack certain HBGAs are resistant to specific strains of norovirus [3].

The human immune response to norovirus is unclear. Up to 90% of people develop antibodies against norovirus before adulthood. However, the presence of antibodies does not necessarily offer protection against norovirus infection. The genome of norovirus undergoes frequent changes. Even small changes in the capsid protein can result in significant changes in the virulence of norovirus. Like the variation in antigenicity of the common cold virus, this antigenic diversity and changeability of norovirus may contribute to the lack of protection from antibodies generated from previous infection by earlier versions of a norovirus strain. The emergence of new GII.4 variants correlates with the occurrence of norovirus outbreaks worldwide.

The detection of norovirus was historically done by electron microscopy. The current gold standard is reverse-transcription polymerase chain reaction (RT-PCR). This molecular method is time-consuming and expensive. No primer set detects all noroviruses because of the huge variation among noroviruses. There are enzyme immunosorbent (EIA) tests currently on the market but the sensitivity of EIA tests is lower than 50% [4].

The outbreaks of norovirus are seasonal. The peak season is January, probably due to frequent human to human contact in closed environments during winter holidays.

Interestingly, the seasonal outbreaks of norovirus and *Clostridium difficile (C. difficile)* disease overlap. In hospital settings, norovirus outbreaks can masquerade as *C. difficile* infection [5]. Diarrhea in patients can be caused by more than one pathogen. In a study of fecal samples collected in emergency rooms, more than one pathogen was found in 9% of the stool samples. Patients suffering from antibiotic-associated diarrhea who test positive for norovirus should still be tested for *C. difficile* infection.

There is no specific therapy for norovirus infection. Hydration is the key to recovery. Anti-diarrheal medicine such as loperamide may be beneficial. A drug named nitazoxamide shortened the disease period in a study but the effectiveness of the drug needs further study. Oral immunoglobin therapy is promising but the outcome is, so far, inconclusive.

The development of a vaccine against norovirus is challenging due to the lack of an animal model for testing infectivity and protection, the huge antigenic variation among genogroups, and the lack of success of growing norovirus in mammalian cell culture. Without an effective vaccine, stopping fecal-oral transmission is the key to disease prevention.

In conclusion, norovirus is the leading cause of diarrhea worldwide. The lack of a small animal model and difficulty in propagating norovirus in cell culture greatly hinder the research on norovirus. The pathology is unclear and the available diagnostic tests are less than satisfactory. With the lack of effective vaccine and treatment, the key to reduce norovirus outbreaks lies in disease prevention. The isolation of symptomatic individuals is crucial in infection control.

Manli Davis

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Recent publications from TechLab

Fecal Lactoferrin as non-invasive biomarker in inflammatory bowel diseases. Drugs of Today 2012, 48(2):149-161, J. Langhorst and J. H. Boone. Potential utilities for lactoferrin measurements include a biomarker for mucosal healing and a noninvasive method for following medical and surgical treatment in patients with IBD.

***Clostridium difficile* prevalence rates in a large healthcare system stratified according to patient population, age, gender, and specimen consistency. Eur J Clin Microbiol Infect Dis. 2011 Dec 14. [Epub ahead of print], J. H. Boone et al.** Significantly different prevalence rates in clinically indicated specimens were observed based on patient population and age, the presence of nontoxigenic isolates in all stratified populations, and fluoroquinolone resistance in ARL 027 and non-027 ribotypes.

Glutamate dehydrogenase is highly conserved among *Clostridium difficile* ribotypes. doi:10.1128/JCM.05600-11. R. J. Carman et al. All isolates and ribotypes expressed GDH at levels 500-fold or more above the minimum detection levels of TechLab's commercial GDH assays.

Toilet paper

I have to admit that when I heard that Simon Cowell of celebrity fame --- you know, *American Idol, America's Got Talent, Britain's Got Talent*, etc. --- used anti-aging tricks, I really wasn't too surprised. After all, Botox is

used by many celebrities and a whole lot of non-celebrities too. And colonic irrigation isn't too far-fetched, although I'm not really sure how it supposedly makes his eyes brighter. But when I saw the bit about using black toilet paper, that one really surprised me. Apparently black toilet paper is pretty trendy, and it's starting to be used more in the hottest restaurants in New York, Milan, Paris, and Madrid. I can tell you that I haven't seen it in any airports and rest stops.

I went online to source black toilet paper and sure enough, you can buy a six-pack for $9.99 from a European company called Renova. The toilet paper is jet black. You can also purchase other colors --- green, yellow, blue, orange, red, and fuchsia. The toilet paper is described as "*Fashionable, Sensual, Sophisticated, Fun, Unique!*" Unique perhaps, but I don't know about the other descriptions --- never really thought of toilet paper as sensual, but maybe I'm being too narrow minded. The paper is colorfast, so it won't look like you've gotten some type of weird tattoo in a really weird location.

The Renova site has a "frequently asked questions about toilet paper" section, but there were no questions available. The site also asked for reviewer's comments about the black toilet paper, and so far, no reviews have been posted, at least on the site that I searched. So if you try the paper, you may want to be the first to write one.

There are contests with cash prizes to determine who can make the best wedding dress out of toilet paper. Charmin sponsors one of these contests, and the dresses are incredible creations --- google Charmin toilet paper wedding dresses to take a look. The reason I bring up wedding dresses is that with black toilet paper now available, tuxedoes can now be created! And with all the colors of paper now available, think of the possibilities for prom dresses! One bit of advice --- wherever you choose to wear your toilet paper attire, be sure to check ahead to make sure it's not going to rain! Don't want to get arrested for indecent exposure!

The upper GI: stomach ulcers

Gastric cancer is a multi-step and multi-factorial disease. The major contributing factor in 71- 95% of all gastric cancers is a bacterial infection of the stomach by *Helicobacter pylori* [1]. *H. pylori* elicits chronic gastritis in some infected individuals, resulting in stomach ulcers. The most common symptoms of stomach ulcers are abdominal pain, heartburn/acid reflux and abdominal discomfort occurring 2-4 hours before or after a meal [2]. Nearly 6,000 Americans die annually from ulcer related complications.

Originally ulcers where thought to be caused by stress or spicy foods, but in 1985 the discovery of a Gram negative, spiral, microaerophilic bacteria known as *H. pylori* was isolated from stomach lesions. This discovery was not considered of public health importance until early 1990's [3]. *Helicobacter* is transmitted via fecal-oral route by either ingesting contaminated food or water. In developing countries with poor sanitation, an estimated 50% of the population is infected in early childhood and nearly 90% by adulthood [2]. Worldwide *H. pylori* boasts a prevalence of 50% [1]. Approximately 1 in 8 people will develop stomach or duodenal ulcers in their lifetime. In the U.S. alone stomach ulcers affect more than 5 million people each year. The use of aspirin or other NSAID pain relievers has also been shown to cause stomach ulcerations [1].

Helicobacter's survival in the harshly acidic stomach environment is based on a suite of enzymes it possesses. *H. pylori* possesses cytochrome oxidase, catalase and, most importantly, urease. Urease allows the bacteria to convert urea to ammonia and carbon dioxide, transforming the stomach pH from 2 to 5 [4]. This reduction creates a significant problem in individuals on proton

pump inhibitors (PPIs) as the acid suppression increases gastric mucosal inflammation which varies depending on the level of suppression [4]. Mucosal inflammation results in the loss of gastric glands causing active chronic gastritis. This progression elevates the risk of developing gastric cancer. Eradication of the bug in patients on long term PPI therapies heals gastritis and prevents the progression to atrophic gastritis, but there is no evidence indicating that this reduces their risk of gastric cancer [1]. Other complications caused by acid suppression from *H. pylori* include iron deficiency, anemia and vitamin B12 deficiency, as well as poor absorption of certain medications such as l-dopa and throxine [1]. Notable virulence factors of *H. pylori* are CagA and VacA with Eastern CagA strains having a much higher virulence than Western strains [1]. Cytotoxin associated gene A (CagA) is responsible for ulcer production by disrupting junctions in the gastric epithelial [1]. Vacuolating cytotoxin gene A (VacA) diffuses urea from the mucosa to the stomach allowing urease to provide energy to the organism.

Diagnosis of *Helicobacter pylori* consists of two strategies: invasive and non-invasive. The most common invasive procedure is endoscopy where biopsy tissue is taken and cultured. The tissues can also be fixed and examined by histology. Other isolation sources include gastric juices, blood and liver tissues [3]. Non-invasive procedures include the urea breath test (UBT), stool antigen test and serology. The urea breath test is the most popular non-invasive test methodology. The principle behind this method is that the urease activity of *H. pylori* will cleave the urea from the carbon isotope allowing the measurement of labeled carbon dioxide to be measured during exhalation using a mass spectrometer [5]. During this test, the fasting patient ingests 50 mg of C13 or C14 tagged urea with breath samples taking before, 15, 30, 45 and 60 minutes after ingestion, with the central measurement at 30 minutes. Other non-invasive tests rely on the presence of analyte in the sample for a positive diagnosis implementing stool antigen, serum antibodies, or molecular probes with *H. pylori* specific markers [1, 5]. Sensitivity between rapid formats of stool and serology tests varies greatly. Some stool antigen tests are as sensitive as UBT [5]. In all non-invasive test formats, there is the possibility of inaccurate results. People on PPIs are reported to have false negative test results. Often patients have to suspend PPI therapy for several days prior to testing to insure accurate test results [1].

Treatment of *H. pylori* consists of antibiotic and PPI therapy, however many strains are becoming resistant to clarithromycin and metronidazole, the two preferred antibiotics. Strains in developing countries especially have a high prevalence for metronidazole resistance (80%) whereas strains in the U.S. have only 20% prevalence [3]. For the time being *H. pylori* has a very low resistance to amoxicillin and almost no resistance to rifampin [3]. Often triple drug therapies consisting of clarithromycin and another antibiotic in conjunction with a high dose PPI are implemented [1]. Follow up testing via UBT or endoscopy, depending upon severity of infection, should be performed within 4 weeks of treatment completion.

Due to the nature of *Helicobacter pylori* infections, proper water treatment and hand washing are the recommended actions of prevention. As with cervical cancer, the best preventative strategy for *H. pylori*-related gastric cancer may be vaccination. However; the challenges associated with vaccine development may cause ulcers on its own.

Heather R. Totty

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Tell-tale Microbes

In the hunt for biomarkers of human diseases, whether they are protein, DNA, RNA, miRNA, or metabolites, researchers often are faced with bacteria biomarker matches. At times these clandestine matches are thrown out because the focus of the research is to find *host* biomarkers; however these bugs might be telling something equally important. The bacteria thriving in your gut can often be correlated with healthy or diseased states (2). Changes in a person's microbiome can have disease inducing (dysbiosis) or disease protective (protiosis) effects (3). Dysbiosis can be an indicator of diseases such as inflammatory bowel disease (IBD), cancer, diabetes, or neurological disorders. Here we explore how the microbiota of your gut changes in concert with, and may even contribute to disease.

So what bacteria does a healthy gut have? The healthy gut generally contains microbes belonging to the Actinobacteria, Bacteroidetes, Firmicutes, and Verruco-microbia phyla. Having a robust and diverse population of *Bifidobacteria* or *Lactobacillus* is seen as especially beneficial because they help the host by stimulating the immune system, producing vitamins, inhibiting pathogens in the gut, reducing blood ammonia and cholesterol levels, and reducing constipation. How do these bacteria help the host? Part of how they help is by producing short chain fatty acids (SCFA) such as acetate, lactate, and butyrate (5). These SCFA are helpful in a number of ways. Butyrate, for instance, is an energy source for colon cells and is associated with the "upkeep" of the gut epithelium.

So, what bacteria are associated with disease? We already know that an increase in toxin-producing *Clostridium difficile* are considered harmful, but (as you will read below) bacteria could act as a cause or marker of diseases such as inflammatory bowel disease, autism, diabetes, rheumatoid arthritis, and others.

The mechanisms that govern the relationship of the gut and its microbiota have been vague. However, a recent *Science* paper has shown that the intestinal protein RegIIIγ helps maintain a physical barrier between the microbiota and the gut epithelium, allowing bacteria to reside in the gut without harming the lining of the host's small intestine. Interestingly, RegIIIγ is seen in higher concentrations in patients with inflammatory bowel disease (IBD) indicating that in these patients there may be a disruption in the protective barrier between the host and bacteria. People with IBD have also been found to have a reduction in the variety of Firmicutes and Bacteroidetes phyla (two of the microbes commonly found in healthy individuals), as well as an unusual immune response against *Candida albicans, Pseudomonas fluorescens* and *Escherichia coli*. Additionally, humans who carry a mutation in the NOD2 gene (nucleotide-binding oligomerisation domain containing 2) - a receptor that can recognize bacterial cell well proteins - have a 1.75- to 4-fold increase risk of developing Crohn's Disease. This makes sense as a similar mutation in NOD2 allows microbial-host interactions that can result in production of immune-stimulating cytokines by intestinal and immune cells in the gut. The resulting immune responses and inflammation mimic Crohn's disease. Germ-free mice with the same NOD2 mutation, however, do not go on to develop Crohn's disease. This has led to the hypothesis that the bacteria in your gut may play a role in your risk of developing IBD.

Bacteria can also play a role in a number of cancers, especially when it comes to those of the digestive system. By now it is almost common knowledge (and this finding was rewarded with the 2005 Nobel Prize) that colonization of the stomach with *Helicobacter pylori* can lead to gastric ulcers. There have also been further studies which indicate that

H. pylori can predispose people to gastric cancers. Recently two independent studies have looked at tumor microbiota taken from patients with colorectal cancer (CRC). Both studies found more *Fusobacterium* in tumor samples compared to neighboring normal tissue. As with the IBD studies, these studies also found that Bacteroidetes and Firmicutes bacteria were depleted in CRC tumors. Cytokine interleukin 10 (IL-10) is additionally associated with CRC progression in humans. Germ-free mice with an IL-10 mutation are resistant to CRC- this resistance is lost when mice are colonized with normal human gut microbiota. Similarly, tumor-prone mice (Apc^{Min}) develop significantly fewer gastrointestinal (GI) polyps in a germ-free environment compared to one that allows for colonization. All these results suggest that the bugs in your gut play a role in the health or disease of your intestines.

The bacteria in your gut don't solely report on the health or disease of your GI tract, but can also be an indicator of disease in many other organs. A strain of non-obese diabetic mice that lack a microbial-recognition immune system receptor (MyD88) are usually resistant to developing type I diabetes. However, when these MyD88-deficient mice are raised in a germ-free environment they develop diabetes. The development of diabetes can be prevented if the germ-free, MyD88-deficient mice are inoculated with microbes to populate their gut.

A number of behavior syndromes, including anxiety and autism, are also believed to be affected by the gut microbiota. Studies in germ-free mice allow neurogastroenterologists to see the effects of adding in or taking away bacteria from the murine microbiota (1). In germ-free mice, the stress hormone corticosterone is increased – this is reversed upon recolonization with *Bifidobacteria* genus. Doctors have found that humans who have received *Lactobacillus helveticus* and *Bifidobacterium longum* treatment not only show a reduction in anxiety but also in serum cortisol levels. Autistic children often suffer from some sort of GI stress- whether it be diarrhea, constipation, bloating, gastroesophageal reflux (GERD) or other ailments. These problems are often believed to be caused by an abnormal gut flora and studies have shown that *Clostridium* and *Ruminococcus* populations differ in autistic children compared to control children.The list of diseases associated with bacteria is numerous (4). Could rheumatoid arthritis be associated with *Porphyromonas gingivalis*? Is Guillain-Barré syndrome (characterized by acute neuromuscular paralysis) initiated from infection with *Campylobacter jejuni*? Why do antibiotics help to calm obsessive-compulsive disorder in children with PANDAS (pediatric autoimmune neuropsychiatric disorders associated with streptococcal infection)? Obviously certain bacteria can initiate a strong immune reaction- especially in genetically predisposed individuals, and in some cases this might be something that could be useful in finding treatments. It is very important to also keep in mind all of the good things that bacteria do for us in preventing disease and aiding in digestion.

There is still much more to learn about the relationship between human health, disease, and microbiota. Why do we see such changes in microbiota? Perhaps it is due to diet, or perhaps certain diseases release metabolites that specific strains of bacteria thrive on. Alternatively, sometimes it is the bacteria that bring disease into the body or may cause disease due to production of their own metabolites. Over time these webs of interactions will become clearer to researchers, who will then be able to utilize the key points of the web to develop new, hopefully effective treatments. Who knows, maybe pro- and pre-biotics for intestinal infections may come to replace antibiotic therapy over time.

Rebecca Easley

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Assessing intestinal inflammation in the pathophysiology of depression

Scientific evidence has been attained that may elucidate the role of intestinal inflammation in the comorbidity of clinical depression [1]. The human brain and intestines communicate and convey messages through the enteric nervous system. This system utilizes over 30 types of neurotransmitters including acetylcholine, serotonin, and dopamine to transfer chemical signals between intestinal neurons and the central nervous system (CNS); approximately 95% of the serotonin and 50% of dopamine produced in the human body is located within the intestinal epithelium which is coated with over 100 million neurons [2, 3]. Without the presence of these neurotransmitters, the digestive system would be unable to send the necessary signals to produce enzymes or absorb critical nutrients and essential amino acids. Thus, this vast network of neural cells can communicate with the CNS as part of a biofeedback system. In this respect, the intestinal tract might be considered a “second brain” in which signals of stress, anxiety, and pain are communicated between two nervous systems [2].

Effectively, chemical signaling between these two bodily systems may function to exacerbate physiological disease processes. As an example, antidepressants cause gastrointestinal distress such as diarrhea, nausea, and constipation in one quarter of all patients [4]. Antidepressants effectually block the release of serotonin in the intestines thereby increasing availability within the central nervous system. In the process of uncovering the circuitry between the two “brains,” scientists are able to better understand emotional psychology from physiological enteric processes [2]. In cases of extreme stress, immunological cells in the intestine secrete histamine and prostaglandins which in turn produce an inflammatory response. As a consequence, these chemicals will cause considerable diarrhea and cramping [2].

In addition to neurochemical connections, lipopolysaccarides (LPS) and administrations of pro-inflammatory cytokines are capable of causing chronic neuroinflammation [1]. The systemic injection of LPS has been shown to increase tumor necrosis factor-α (TNFα) concentrations for up to 10 months; TNFα, a pro-inflammatory cytokine, may be implicated in behavior complexes similar to those observed in major depressive episodes [1]. From these studies, several scientists have sought to ascertain if a link exists between depressive symptoms and immunological responses to intestinal inflammation [2, 4].

Recently, a study from Maes et al., 2012 examined serum concentrations of IgM and IgA against LPS of gram negative enterobacteria from 112 depressed patients and 28 normal control patients. Results of the study suggest a heightened immune response against LPS in depressed patients as compared to a control group [1]. This research also implied that bacterial translocation across epithelial tissues from the intestines may be a primary factor in instigating the onset of depression in psychologically vulnerable individuals [1, 5]. Additionally, translocation of commensal bacteria may play a role in the perpetuation of the inflammatory response as a secondary factor to overall systemic inflammation.

Subsequent investigations have suggested possible methodologies to mitigate both the inflammatory response of the intestinal epithelium and symptoms of depression by the introduction of probiotics such as *Lactobacillus rhamnosus* [4]. Ingestion of probiotic enterobacteria attempts to mediate toll like receptor (TLR) signaling from the enteric nervous system to the central nervous system to minimize inflammation. While the exact mechanism of probiotics is not completely understood, experimental data from animal models have shown reduced

anxiety levels, lower proinflammatory cytokine concentrations, and overall improvements in nutritional status [4]. These studies highlight the need of clinicians and researchers to better understand the physiological interactions between the intestines and the CNS. Additional insight on this biofeedback system may provide physicians with valuable alternative treatments for gastrointestinal diseases associated with neurological dysfunction.

Krista Williams

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