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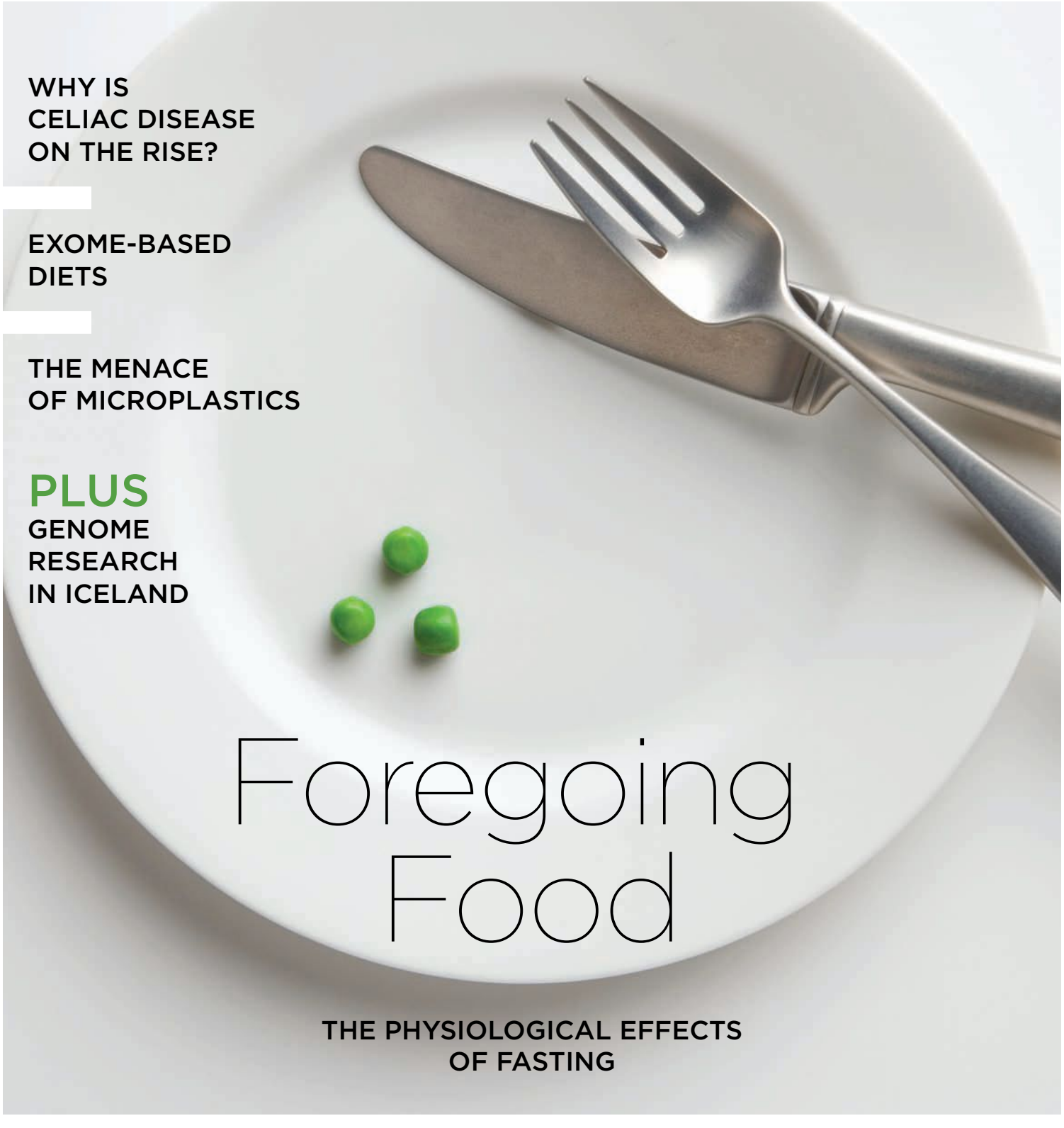

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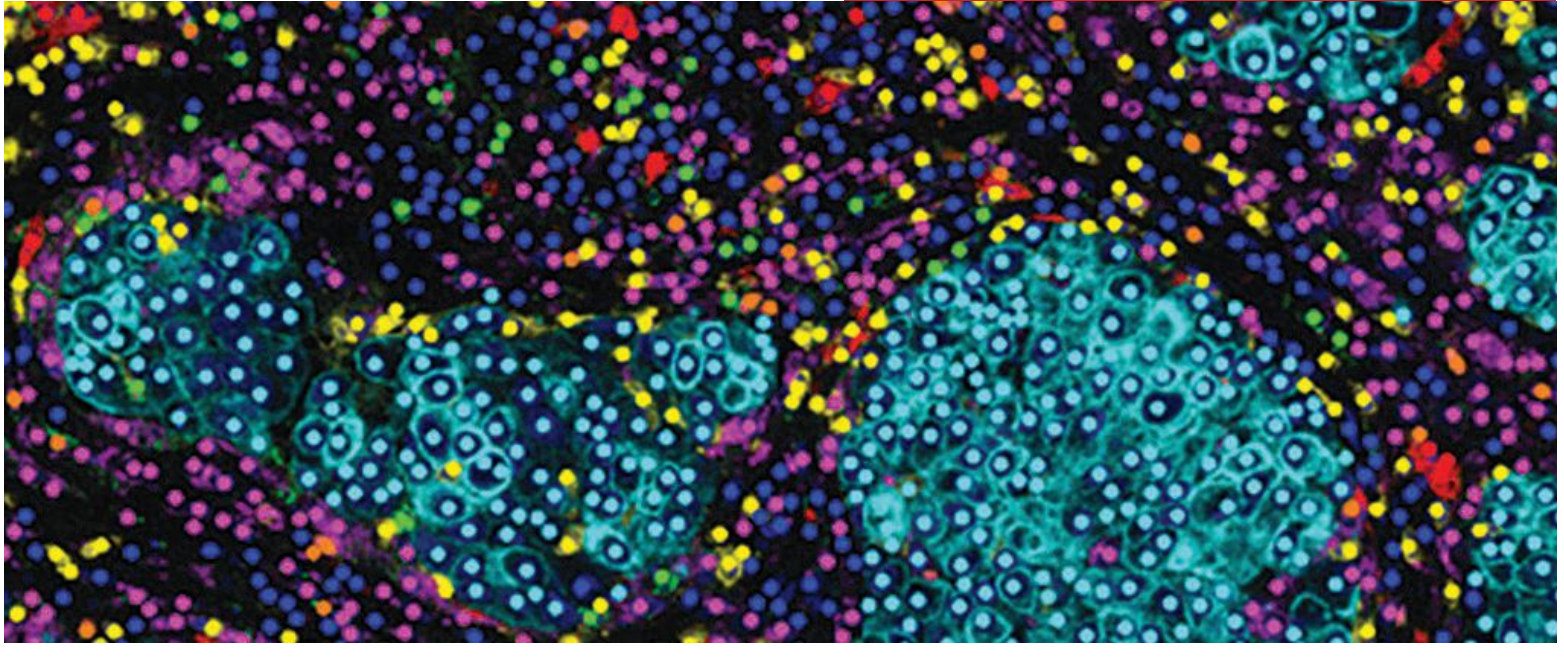

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Speaker List

Adelaide Rhodes, PhD – Oregon State University
Rob Knight, PhD - University of California
Ahmad M. Khalil, PhD – Case Western Reserve University
Benjamin Glick, PhD – University of Chicago
Costin Antonescu, PhD – Ryerson University
Prashant Mali, PhD – University of California San Diego
James Eberwine, PhD - University of Pennsylvania
Elizabeth Hillman, PhD – Columbia University
Traver Hart, PhD – University of Texas
Holly Aaron, MS – University of California at Berkeley Cancer Research Laboratory
James Taylor, PhD – Johns Hopkins University
Maria Lehtinen, PhD - Boston Children's Hospital Harvard Medical School
Maroof M. Adil, PhD – University of California at Berkeley
Neena K. Pyzocha, PhD - MIT
Kris Wood, PhD – Duke University
Manolis Kellis, PhD - MIT
Anita Bandrowski, PhD - University of California, San Diego
Lanlan Shen, PhD - Baylor College of Medicine
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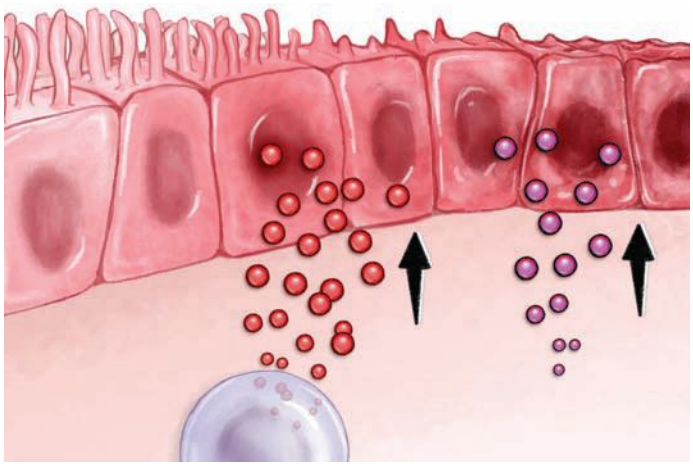

Features

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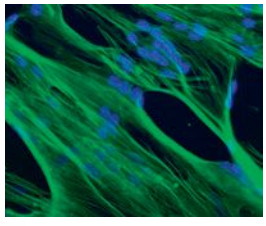

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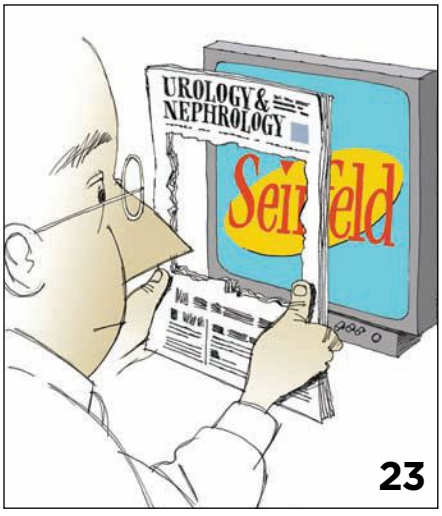
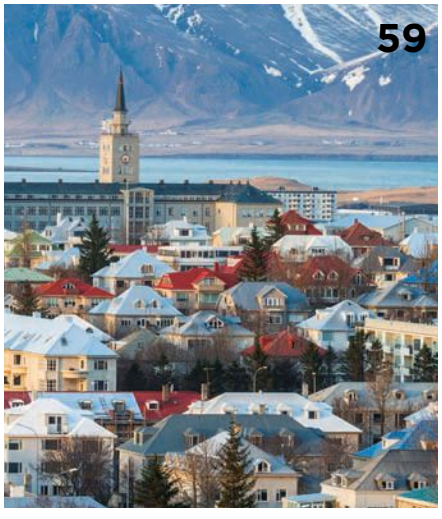


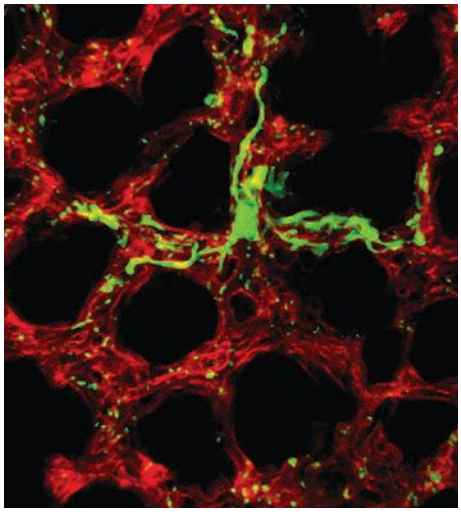


CORRECTIONS:

In "Passing the Buck" (*The Scientist*, May 2017), the location of Aarhus University was mistakenly identified as the Netherlands. The university is in Denmark.

The Scientist regrets the error.

Online Contents



THIS MONTH AT THE-SCIENTIST.COM:

VIDEO
Snake Secrets
See a boa species that scientists hadn't witnessed alive in more than 50 years.

VIDEO
Lung Blood
Watch the birth of platelets in the lungs of mice, a finding that contributes to a new understanding of the respiratory organ's role in blood formation.

VIDEO
Microbiome Racer
Genomicist Lauren Petersen wins a mountain bike race after she altered her own gut microbial communities.

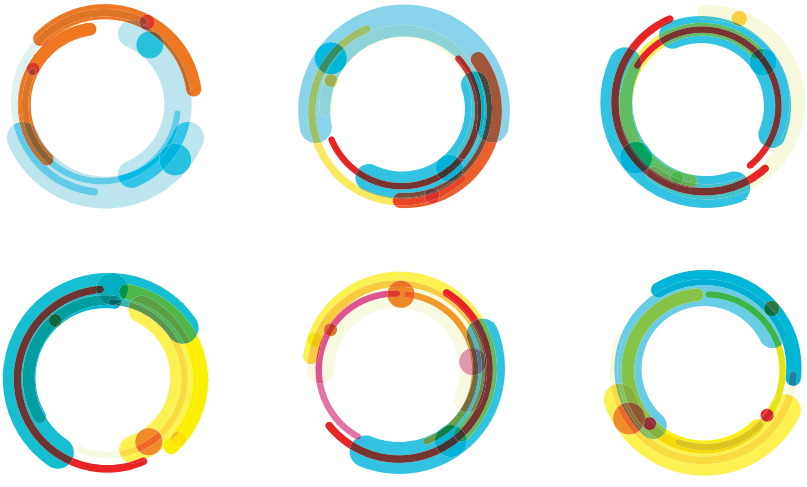
AS ALWAYS, FIND BREAKING NEWS EVERY DAY, AND LEAVE YOUR COMMENTS ON INDIVIDUAL STORIES ON OUR WEBSITE.

Coming in July/August

HERE'S WHAT YOU'LL FIND IN NEXT MONTH'S ISSUE:

- Circular RNAs
- Building 3-D nanostructures with DNA
- Endocannabinoid signaling
- New tools for studying mechanobiology
- Predatory journals

AND MUCH MORE



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Contributors

Ee Ling Ng was born and raised in Malaysia. Before starting her undergraduate studies at Monash University Malaysia, Ng spent some time doing conservation-related volunteer work around the country. "I think growing up in Malaysia and doing the volunteer work opens your eyes to how many problems we have in the environment when we blindly chase economic growth," she says. Later, Ng spent two years working on a master's in applied ecology, studying and conducting research at four different universities in Europe. During one of her placements, at the University of Coimbra in Portugal, Ng was introduced to soil ecology when she met José Paulo Sousa, a professor of soil ecology and ecotoxicology. "That's when I realized soil was one of the most complex ecosystems in the world, and that was very exciting," Ng says. Ng studied soil science during her doctoral studies at Monash University in Melbourne, Australia, where she currently resides, working as the manager of the Australia-China Joint Research Center: Healthy Soils for Healthy Food and as a research fellow at the University of Melbourne.

Read Ng's feature about plastic pollution on page 40.

"I grew up in Brooklyn, back before it was trendy," says **Robert Sapolsky**. From an early age, Sapolsky knew he wanted to study primates—he started writing fan letters to primatologists at age 12 and studied Swahili in high school to prepare himself for future field work in East Africa. But during his freshman year at Harvard University, the primatology class he wanted to take got canceled, so he enrolled in an introductory neuroscience course instead. "[It] blew me away," he says. "Ever since, I've been alternating between being a neurobiologist, studying the neural bases of behavior, and a primatologist, studying the evolutionary bases [of behavior]." Sapolsky says his career has had a number of defining moments, including "the first time I saw baboons in the wild" and when he realized that "glucocorticoids make neurons more vulnerable to all sorts of neurological insults." During graduate school at Rockefeller University, Sapolsky started writing about science for the lay public. He has penned a number of books since, but his personal favorite is *A Primate's Memoir*. "It's about my three decades of research on baboons," he says. "And it's a great reminder of my time spent out in the field."

In an essay based on his new book *Behave* (page 63), Sapolsky writes about the role of the human brain's insula in feeling metaphorical disgust.

George Retseck is an artist with a proclivity for science. He has been a freelance illustrator since 1980, after graduating from Kutztown University in Pennsylvania with a degree in communication design. As a child, he always loved art, but was also drawn to science, especially if there was some sort of drawing project involved. His great-grandfather was an artist and a source of inspiration; Retseck recalls his classic painting of a deer in a forest hanging in his aunt's living room.

Retseck calls himself a "nuts and bolts type of illustrator," specializing in all things science and technology, and he has been a prolific contributor to *Scientific American* and *Popular Mechanics*. He acknowledges that freelancing is not always easy and can be lonely at times, but it has afforded him the freedom to raise a family and to work from campgrounds to coffee shops in Europe. "Wherever I can get an Internet connection," he says. "That's been pretty nice."

Retseck has illustrated *The Scientist*'s Modus Operandi column since its print debut in July 2011. This award-winning column highlights a newly published scientific method by pairing a description of the technique with an explanatory infographic. Retseck's art for this month's article appears on page 25. You can see all of his drawings by clicking on Modus Operandi under "Magazine" in the website's navigation bar.

STUART WINTHROPE, UNIVERSITY OF MELBOURNE; THOMPSON MCCLELLAN; GEORGE RETSECK

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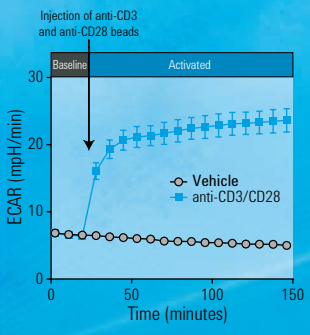

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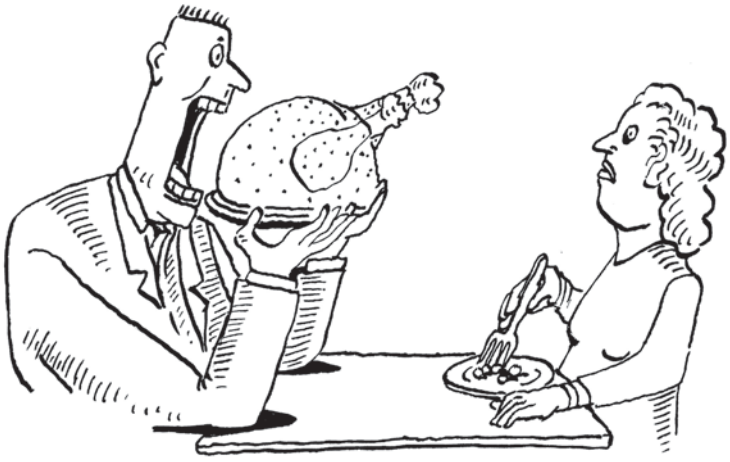
Is Less More?

Diets: From art to science

BY MARY BETH ABERLIN



ANDRZEJ KRAUZE

Still life is an art form whose popularity stretches back to the roots of human civilization. Depictions of food often feature prominently in wall paintings in ancient Egyptian tombs and are common in Roman mosaics and frescos. By the 16th century, Dutch and Flemish artists were producing incredibly lush still lifes, some almost over-the-top in their portrayal of food as a corporeal pleasure. Artists of more recent centuries cubed, Dada'd, abstracted, and photorealismed the genre's subject matter.

For this month's cover we chose what I view as a very austere still life, but it is an apt illustration for Senior Editor Bob Grant's feature (page 26) on the science behind fasting—what happens to the body's physiology that is detrimental or beneficial. Evidence suggests that a steady restriction of caloric intake extends life span and provide a host of health benefits. But adherence is almost impossible for most people. Grant digs into the science behind fasting diets and reports that the regimens may be enjoying fad status these days simply because they cut calories in a more compliance-friendly way. Is it possible to fast and lose, or are such diets doomed to the fate of the grapefruit diet, the blood-type diet, and the cookie diet?

Gluten-free diets are another fad, but are currently the only cure for those with celiac disease. Catherine Offord describes the worldwide rise in the number of people diagnosed with the autoimmune disorder and examines some of the possible contributing factors (page 33), given that currently known genetic risks don't seem to explain the phenomenon. One challenge facing epidemiologists is the multitude of gluten-free dieters who have self-diagnosed gluten sensitivity, rightly or wrongly.

Two of this month's Notebooks deal with how humans process what goes down their gullets. "Chewing It Over" (page 17) reports on evolutionary changes to mammalian lower jawbones that, together with changes in dentition, ultimately allowed mammals to process a wider variety of foods. A genomicist who is also a competitive cyclist did a DIY repopulation of her gut microbiome after years of antibiotic treatment for chronic Lyme disease. Buoyed by the results, she has gone on to study the gut microbiomes of athletes to suss out exercise's effects on microbial health ("The Sports Bug," page 18).

The lead article in The Literature department (page 48) features work done with fruit flies and mice that were fed a diet based on the overall amino acid ratios determined from sequencing the animals' protein-coding regions, the exome. Not only did the experimental subjects prefer the exome-matched diet, they ate less and grew faster.

Other articles in this food-focused issue include a Foundations piece about the discovery of IgE, notorious for its role in food allergies, and a profile of UC Davis's Lindsay Allen, who has devoted decades to researching the importance of consuming an adequate amount of micronutrients, especially vitamin B12, and has developed a method for assaying the B vitamin–content in human breast milk (page 50). Another UC Davis professor is our Scientist to Watch. A farmer's daughter, Amélie Gaudin studies environmental stress and root ecology with the goal of improving crop yields (page 53).

Soil ecology and farming practices are an important part of "Planet Plastic" (page 40), a feature by Ee Ling Ng, a scientist who manages a research center devoted to the promotion of "healthy soils." Describing the massive inundation of plastic pollutants, Ng drills down on the nano- and microplastic particles found in consumer products and that result from the degradation of larger plastic debris. The opening photo will give you pause: Chinese fields covered to the horizon in rows of plastic sheeting, meant to encourage crop growth in areas that are less than ideal for farming.

Pottery from ancient Greece often featured depictions of food, so I'll leave it to a man of that time, Hippocrates, to enunciate its importance: "Let food be thy medicine, thy medicine shall be thy food." ■

Editor-in-Chief
eic@the-scientist.com

Speaking of Science

I am the son of a refugee from Nazi-occupied Europe and the grandson of refugees from tsarist Russia. I [am] marching in honor of the immigrants who came to this country and contributed to the advancement of scientific knowledge and to improvements in health that have benefited the people of Boston and the citizens of this planet.

—Harvard Medical School researcher **Thomas Michel**, about why he planned to participate in the April 22 March for Science in Boston (April 21)

To get this many thousands of scientists in so many towns and cities around the world to say, "We should go public with our science and with our concerns about the future of science," that is something I've never seen—at least in half a century.

—**Rush Holt**, CEO of the American Association for the Advancement of Science, on the March for Science movement, which included hundreds of marches across the U.S. and around the world (April 22)

Today, I was Trumped. I have had the pleasure of serving on the EPA Board of Scientific Counselors, and my appointment was terminated today.

—Michigan State University environmental economist **Robert Richardson**, in a tweet following his dismissal and that of 8 of his colleagues on the 18-member US Environmental Protection Agency's Board of Scientific Counselors, who received notification that they would not be serving a second three-year term, as has been customary (May 5)

Questions about the validity of forensic science will not go away. Failure to address them will lead to further convictions of innocent people. For our society, the stakes don't get much higher.

—**Sunita Sah, Arturo Casadevall, Suzanne Bell, S. James Gates Jr., Thomas D. Albright, and M. Bonner Denton,** authors of an opinion piece in *Scientific American* about the decision by Attorney General Jeff Sessions to shutter the National Commission on Forensic Science (May 8)

KERRY GRENS

They vary a lot, but what they have in common is, if passed, they would all tend to undermine the integrity of science education. That's why we're against them, science teachers are against them, school boards are against them.

—**Glenn Branch,** deputy director of the nonprofit National Center for Science Education, on a new crop of bills that aim to change the way several scientific topics are taught in US public schools (May 8)

French science . . . would not survive a withdrawal behind our frontiers and restrictions to the circulation of brains and ideas. On an endless number of topics, [including] migration, health, the environment, and even the history of our country, the ideas disseminated by the National Front are in open contradiction with undeniable evidence established by research and with the necessary autonomy of the scientific community.

—**The directors of nine of France's major public research institutes,** in an open letter circulated before the French presidential election in early May. National Front candidate Marine Le Pen lost to centrist Emmanuel Macron in a landslide. (April 27)

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Notebook

LÍVIA CORRÊA

SNAKE, CHARMER: Biologist Bruno Rocha shows the rare boa, *Corallus cropanii*, to local children in Guapiruvu, the village closest to where the snake was found.

The Half-Century Snake

Earlier this year, wildlife biologist Bruno Rocha received the call he'd been dreaming of his entire adult life. Two men from the small village of Guapiruvu, southwest of São Paulo, Brazil, had captured a snake that they said looked exactly like the one in the brochures Rocha had distributed in the area a couple of months earlier. Rocha, a researcher at the Butantan Institute, jumped into his car and drove the more than 200 kilometers that separate São Paulo from Guapiruvu. Upon arrival he confirmed, to his amazement, that the snake was indeed a *Corallus cropanii*, one of the world's rarest boas. Standing before the exceedingly rare snake, Rocha became the first biologist to lay eyes on a living specimen since 1953, when the species was first described.

Ever since 1953, researchers had been trying unsuccessfully to get hold of another live Cropan's boa. For the past century, the Butantan Institute has received snake specimens from all over Brazil. Yet, among thousands of preserved snakes floating in the liquid-filled jars lining the shelves of collection rooms at the institute, *C. cropanii* is poorly represented—the institute only has one specimen. And there are just a handful of others held at other institutions. All known specimens of the boa came from a region of São Paulo state called Vale do Ribeira, listed by the UNESCO as a World Heritage site (now called Atlantic Forest South-East Reserves and including part of the state of Paraná) for having one of the largest continuous stretches of the Brazilian Atlantic Forest, a highly threatened biome and biodiversity hotspot.

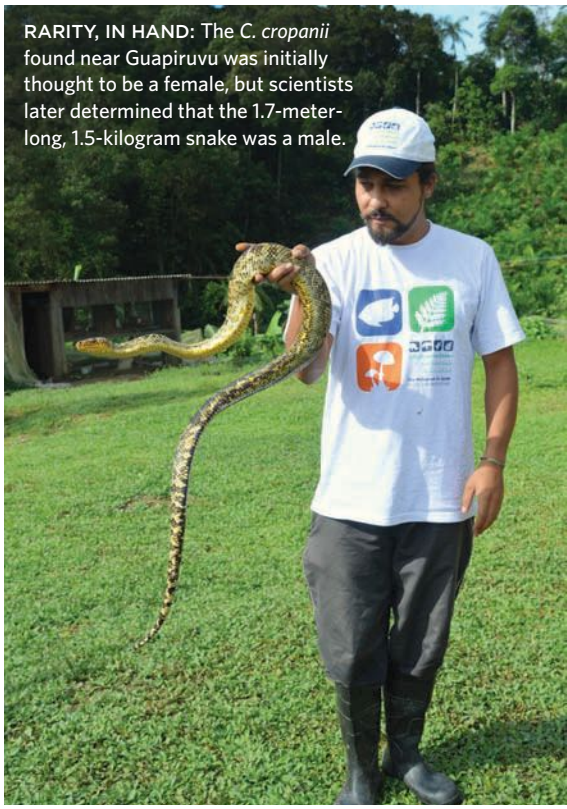
Among the reasons why researchers have not had access to living specimens of *C. cropanii* for so long is that, although boas are not venomous, people who live in the same forests as the snakes tend to kill them on sight. To enhance his chances of finding a living specimen of the rare boa, Rocha decided to enlist the help of

the locals in finding and collecting the snake—populations of which are likely extremely rare, though researchers have never been able to conduct a thorough census. He got funding from the International Union for Conservation of Nature and, with the aid of some colleagues and a local association, "Amigos da Mata," he set up an environmental education event in Guapiruvu late last year.

For the first part of the event, Rocha listed some of the 18 species of snakes that inhabit the region and informed attendees how to identify venomous ones. Then, he introduced *C. cropanii*.

We know next to nothing about the Cropan's boa's natural history: its habitat and how it uses the habitat, diet, foraging behavior, activity patterns, et cetera.

—Robert Henderson
Milwaukee Public Museum



RARITY, IN HAND: The *C. cropanii* found near Guapiruvu was initially thought to be a female, but scientists later determined that the 1.7-meter-long, 1.5-kilogram snake was a male.

"I told them that it's a special animal, that this is the only place where it lives," Rocha says. "And that if people continued to kill it, it could go extinct before we learn anything about it."

He was careful to stress the scientific relevance of the reptile above its economic value, because Rocha knew that the species would be attractive to snake smugglers seeking to make a quick buck by selling live boas to the pet trade. After the event, he distributed brochures with a photograph of *C. cropanii* and his contact information in case someone spotted it.

Just two months later, Rocha's approach proved successful. The two men who eventually found the *C. cropanii* said they were about to kill it when one of them recalled the snake from the brochures. So they decided to capture it and call Rocha.

Francisco Franco, a herpetologist who also works at the Butantan Institute but was not involved in the search for *C. cropanii*, says that the rare species cropping up now is the result of more than just chance.

"Rocha elaborated a project, decided to use a specific methodology, and obtained a result," Franco says. "That is not luck, it's scientific merit."

Six years ago, Franco and his team described the fifth known specimen of *C. cropanii*—a dead snake that someone had photographed—and the first one found in Guapiruvu.

Due to its scarcity in herpetological collections, researchers know little about the ecology and behavior of *C. cropanii*. The new live specimen showed "great dexterity" in climbing when placed on a tree trunk, and Franco's educated guess is that the species lives in the forest canopy, hidden from human eyes. "All the individuals reported so far were found on the ground or in easily accessible places, which contradicts this idea," he says. "However, it could be one of the reasons why there are so few records."

Robert Henderson, curator emeritus of herpetology at the Milwaukee Public Museum and one of the world's most renowned experts on boas, shares his colleagues' enthusiasm about the find. Henderson says he thinks that the new specimen will be valuable for shedding light not

LÍVIA CORRÊA

only on the habitat the boa prefers, but also on other aspects of the species' biology.

"Is it an active or an ambush forager, or does it use a combination of strategies?" Henderson wrote in an email. "Assuming it's nocturnal, where is it spending daylight hours?" Rocha and his colleagues are trying to garner more insights into the habits of *C. cropanii* by tracking the newly found specimen in the wild, using a custom-made radio-transmitter attached to the animal.

"Having only one individual, we can't afford losing it," Rocha says. "We needed a device with a long-lasting battery and a strong signal."

The boa's release was slightly delayed, both by the development of the specialized tracking tag and by snake biology—Rocha's team had to wait until the boa shed its skin to glue the transmitter to its freshly emerged scales. On top of that, Rocha caught malaria.

"This is what happens when you spend too much time in the forest, trying to emulate the old naturalists," he jokes.

Villagers in Guapiruvu continue to play an important role in the scientific project on *C. cropanii*. They took care of the specimen in an enclosure in the village before its release. Rocha hopes to continue this fruitful collaboration as the project unfolds. He says he would like to employ the two men who found the *C. cropanii* and is considering the possibility of utilizing crowdfunding to pay them. Rocha is also collecting anecdotes from other locals who, after seeing the live boa in the news or at the shelter in Guapiruvu, are recalling previous encounters with the species.

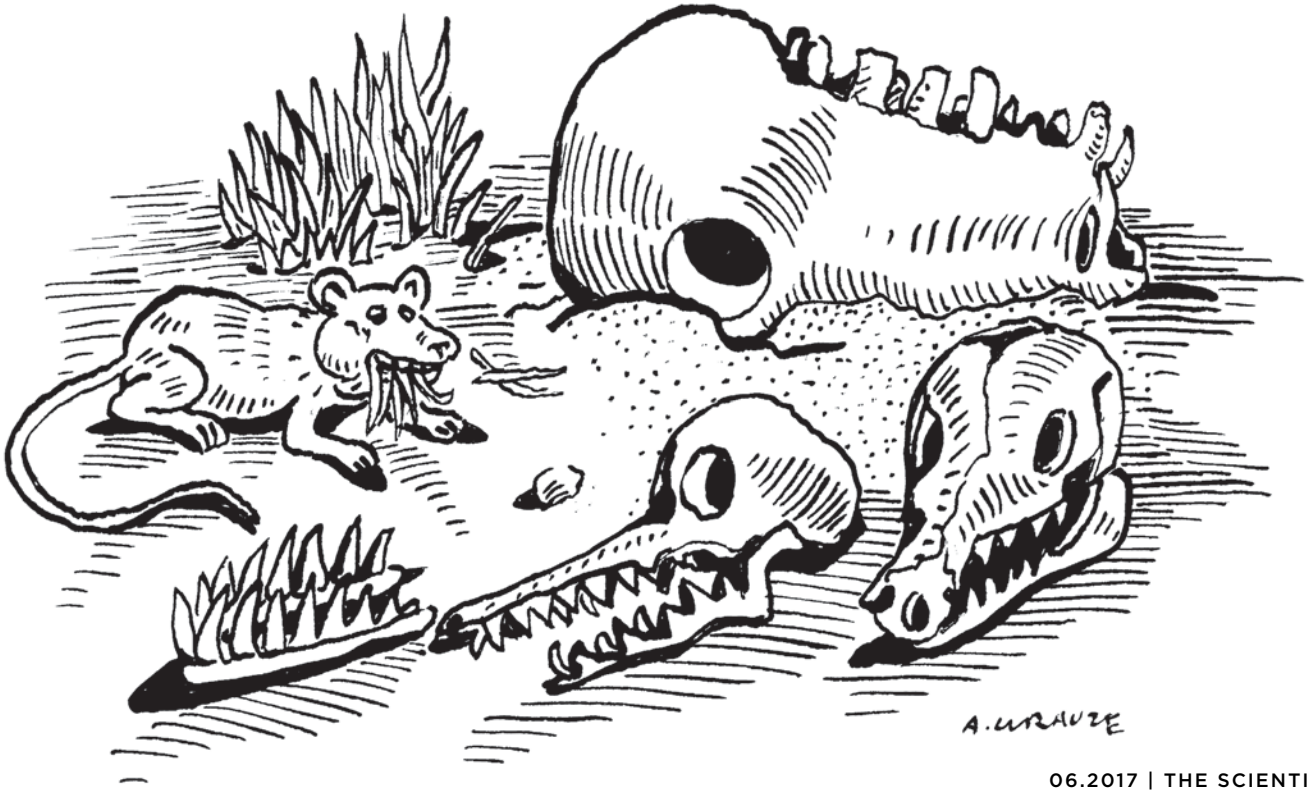
"We know next to nothing about [the Cropan's boa's] natural history: its habitat and how it uses the habitat, diet, foraging behavior, activity patterns, et cetera," Henderson says. "It will be very exciting to learn of Bruno's discoveries regarding this mysterious and elusive boa." —Ignacio Amigo

Chewing It Over

As a master's student at Indiana University a few years ago, David Grossnickle became fascinated with the evolution of early mammals. His dissertation project involved comparing the skulls of modern mammals to those living when the lineage first appeared in the late Triassic, a little more than 200 million years ago, and inferring the ancient creatures' diets based on their jaw shape. But it was while working on this project that Grossnickle came across something that puzzled him.

At the back of each side of extant mammals' lower jaw is a bony protrusion called the angular process. "It's found in basically all modern mammals, so I was using it in all my measurements," Grossnickle explains. "But when I looked at the fossils, I recognized that some of these early mammal groups just didn't have an angular process. That really baffled me, because there are major muscles that attach at the back corner of the jaw."

These muscles are crucial to the way we and our evolutionary relatives chew. Unlike almost all other modern animals, mammals can move their jaws in multiple dimensions to produce not only pitch rotation—the up-down movement allowing cutting of food—but yaw rotation: the side-to-side, grinding motion typical of chewing in cows or goats. This unique physiology gives mammals the ability to process a diverse range of foods—from insects and meat to tough plant matter. Indeed, some researchers consider this



ANDRZEJ KRAUZE

to be a key contributor to our lineage's survival through the mass extinction event at the end of the Cretaceous, which all but wiped out other vertebrate groups such as the dinosaurs.

Realizing that changes in jaw shape must have played an important role in these dietary adaptations, Grossnickle decided to pursue a PhD on the topic at the University of Chicago, and began scouring the literature for information about jaw shape in mammals living from the Triassic through the early Cretaceous. There wasn't a whole lot to find. While many studies of teeth from the same period found that mammalian molars—the flattened teeth at the back of the mouth—were beginning to show small basins allowing the upper and lower teeth to grind against each other and crush tougher food, there had been little exploration of what might have been going on in the jaw to accompany these changes.

"The jaw typically gets ignored," explains Brian Davis, a researcher at the University of Louisville School of Medicine who studies jaw and dental morphology in early mammals. "Teeth are most of the [fossil] record, and they're kind of the business end of chewing, so they get most of the attention," he says. "But jaws are along for the ride on every aspect of this, and they're changing through mammal history too, so quantifying the shape of the jaw is really important."

To make this quantitative account, Grossnickle needed, of course, jaws. But fossilized skulls are rarely in good condition, making detailed measurement a challenge. "We don't have all the bits of the fossils and often they're not even preserved in full three-dimensional form," explains Alistair Evans, a paleontologist at Monash University in Melbourne, Australia. "Often we get jaws that have been squashed flat, and all we see is a sort of photocopy of what the jaw used to be."

So Grossnickle had to take an indirect approach. For starters, he tried to glean as much as he could from published images of fossils to estimate the average dimensions of early mammalian jaws, and in particular the angular process—if it was present at all. He also took advantage of a fellowship at the Field Museum of Natural History in Chicago, which has its own collection of modern skulls—including those of small insectivorous creatures that are considered good analogs for early mammals, such as moles, shrews, and hedgehogs. "I looked through a lot of modern mammals and photographed the jaws from different angles," Grossnickle says. "Then, based on my measurements, I recreated 3-D models."

It's a very interesting study, because it connects the tooth morphology with jaw morphology, which really is something that hasn't been done before.

—Thomas Martin
University of Bonn, Germany

With these models of early mammalian jaws, plus approximations of where muscles might have attached to them, Grossnickle was able to reconstruct how our distant ancestors might have chewed. He found that as mammals evolved a more prominent angular process at the back of the jaw, they lost torque in the up-and-down movement that allows biting. But at the same time, they gained a mechanical advantage in the side-to-side jaw movement that would allow teeth to grind against each other—an adaptation that Grossnickle suspects would have been a critical precursor to changes in teeth morphology that allowed the adoption of a wider range of diets (*Sci Rep*, 7:45094, 2017). "You may first have to have had this ability to produce yaw before mammals would have evolved the molar morphology that requires this motion," he says.

Although this shift towards yaw rotation isn't a surprise given the way modern mammals chew, the link that this study makes between jaws and teeth provides a new piece of the puzzle of mammalian evolution, says paleontologist Thomas Martin of the University of Bonn, Germany. "The point he makes is that changes in jaw geometry took place in concert with the evolution of the molars," he says. "It's a very interesting study, because it connects the tooth morphology with jaw morphology, which really is something that hasn't been done before."

Grossnickle "did a novel job combining teeth and jaws," agrees Davis, adding that "he was able to map really nicely changes in jaw shape across a mammal family tree." Davis notes that future work could include an investigation of further "important landmarks" of the jaw in addition to the angular process, as this bony spike "is not the only point where muscles are attached."

For now, Grossnickle is focused on trying to finish up his PhD. But he hopes one day to take a similar approach to jaw reconstruction in mammals at different stages of the evolutionary timescale. "I looked at the very early lineages of mammals," he says. "But you could apply these methods to other fossil lineages that may be a little more closely related to modern groups. That'd be fun."

—Catherine Offord

The Sports Bug

Genomicist Lauren Petersen has been racing mountain bikes since she was 14 years old. But throughout her teens she battled chronic Lyme disease, suffering recurring bouts of illness that sometimes kept her off her wheels. "I'd feel like crap for a month or two, and then the antibiotics would make me feel like crap, and then I'd rebound a little bit and be okay for a while," she recalls. "It was continuous peaks and valleys."

For seven years, Petersen's doctors prescribed her a barrage of antibiotics. In 2003, at age 21, she took two or three broad-spectrum antibiotics at a time for an entire year, a regimen that she says seemed to finally kick the Lyme. But she wasn't well. "Even when I wasn't sick anymore, I had chronic fatigue and bad stomach issues."

She saw several doctors about her issues, but all the tests probing her immune system, liver function, and more came back normal. It wasn't until she was studying pathogenic bacteria as a PhD student at the University of New Hampshire that she started hearing about the microbiome, and how it might affect health—and how antibiotics can kill the good bacteria in the body along with the bad.

MICROBIOME RACER: Lauren Petersen competing in a mountain bike race in 2016.

JEB WALLACE-BRODEUR

"It kind of rang a bell," says Petersen, thinking back to the many courses of antibiotics she had endured. "I thought, 'Wow, maybe there's something wrong with my microbiome.'"

In 2013, she attended a Gordon Research Conference where microbiome researcher Rob Knight of the University of California, San Diego (UCSD), gave a talk about the American Gut Project, which invites people to send in stool samples for analysis. The project combines data from those samples with the participants' answers to survey questions about disease history, lifestyle, diet, and more, to determine the most important factors in shaping a person's microbiome. "As soon as Rob got there and gave his spiel, and said, 'Get your own microbiome sequenced for $89,' I signed right up."

Petersen recalls the test results she got back later that year: "I had a horrible microbiome—very, very bad." All those years on antibiotics, she says, "I had no idea that essentially my entire gut microbiome was being wiped out." Not only did she lack many of the beneficial bacteria commonly harbored by people without symptoms of disease, but the results also showed that her gut had been colonized by several pathogenic strains that she'd worked with in her lab.

Petersen determined a fecal transplant was the way to restore a healthy microbiome. But because she wasn't infected with a nasty pathogen called *Clostridium difficile*, for which fecal transplant had become accepted therapy, she couldn't find a doctor who would agree to do the procedure. So in February 2014, with the support of her family, she recruited a donor and did it herself. "I just did it at home. It's not fun, but it's pretty basic. It costs like six bucks to do." (The $6 being for the drugstore enema kit.)

The do-it-yourself solution worked. "Within two months I was a new person," Petersen says. "I had no more fatigue. I could ride my bike hard three days in a row, no problem." She started racing four months after her fecal transplant, and was winning races at the pro level soon after that. "Everything changed," Petersen says.

After defending her doctoral dissertation, Petersen started a postdoctoral fellowship in George Weinstock's microbiome research group at the Jackson Laboratory for Genomic Medicine in Farmington, Connecticut. When Weinstock asked what she wanted to work on, she told him she wanted to study athletes. Not only was Petersen an athlete herself, she had intentionally selected an athlete to serve as her fecal donor. And she wondered, "If I had done the fecal transplant with a nonathlete, would I feel this good?" (She had her microbiome reanalyzed after the transplant, and also sent in a sample from her donor; the microbiomes lacked pathogenic strains, and the beneficial strains they contained were identical.)

Petersen quickly rounded up 35 of her cyclist friends to participate in the project, aptly dubbed the Athlete Microbiome Project. She had each submit stool sam-

ples, from which she sequenced the 16S RNA to identify the species that made up each person's gut microbiome and examined the mRNAs present to get a sense of how the individuals' microbiomes might be functioning differently.

Petersen found that athletes had more diverse microbiomes than healthy nonathletes. In particular, many of the cyclists had an abundance of *Prevotella* bacteria, a genus that is often absent from typical American or European gut microbiomes. The cyclists also had a higher abundance and activity of *Methanobrevibacter archaea*, which consume by-products of bacterial fermentation of complex carbohydrates. "It kind of removes [the by-products] from the community, which allows your entire gut microbiome to work more efficiently," Petersen explains. She's submitted the results for publication, and the manuscript is currently under review.

Prior to Petersen's research, scientists in Ireland published a pair of papers on professional Irish rugby players at the request of the Irish Rugby Football Union. Like Petersen, Orla O'Sullivan, a computational biologist at the Teagasc Food Research Centre in County Cork, and her colleagues found significantly more-diverse microbiomes among the athletes (*Gut*, 63:1913-20, 2014). They did not, however, report the presence of any *Prevotella*, Petersen notes, "but they did have lower *Bacteroides* and higher *Akkermansia*, which we also find in our athletes."

This year, O'Sullivan and her colleagues published a follow-up study using metabolic phenotyping and metagenomic analysis to further explore the functional impacts of these microbial differences (*Gut*, doi:10.1136/gutjnl-2016-313627, 2017). But it's still too soon to say why the differences exist. The amount of protein eaten and the level of creatine kinase, a proxy for exercise, were the only two factors that correlated with microbial diversity. "It's an adaption to a lifetime of this kind of training that's maybe having the effect. . . . The diets definitely have a huge role to play in this [as well]," speculates O'Sullivan, who wants to expand the study into other sports. But, she adds, "it's all associations; we haven't figured out the cause."

Under the direction of Embriette Hyde, a research scientist in the Knight lab at UCSD, the American Gut Project is also probing the link between the microbiome and athletics. So far, Hyde and her colleagues have sampled about 80 collegiate athletes at the university, and Hyde says they are beginning to branch out to club sports as well. Petersen is also extending her research. She's got stool samples from more cyclists, collected daily starting a day or two before a race and ending two days afterward, "to see a glimpse of the microbiome in the rested state, during race, and during recovery," she says. The analysis is just beginning, she adds. "I can't tell you too much, but there is some really cool data."

—Jef Akst

Cool Babies

Until a little more than a decade ago, doctors had few options to treat newborns whose brains were deprived of oxygen or blood at birth, a condition known as perinatal hypoxic-ischemic encephalopathy, or HIE. If babies could be stabilized and kept breathing, physicians and nurses could offer only supportive care and had to watch and wait to see how much brain damage their patients would suffer. "This was a disease where we had no treatment that worked, and [around] 60 percent of these babies were either dying or had a disability," says Rosemary Higgins, a program scientist at the National Institute of Child Health and Human Development.

In 2005, research findings reshaped the field. Higgins and other neonatologists reported the results of a couple of large clinical trials testing the effects of so-called cooling therapy on brain damage. Hundreds of babies suffering from HIE—the effects on the brain of oxygen deprivation during delivery, due to umbilical cord problems, the placenta coming away from the uterus too soon, or other complications—had their temperatures chilled from roughly 37 °C to about 33 °C for 72 hours, then slowly rewarmed (in one study it was whole-body cooling, in the other it was just the head). Although many babies still died of the brain damage or ended up with a severe disability, more fared better in the treatment groups than in the control groups (*New Engl J Med*, 353:1574-84; *The Lancet*, 365:663-70).

> This was a disease where we had no treatment that worked, and around 60 percent of these babies were either dying or had a disability
>
> —Rosemary Higgins, National Institute of Child Health and Human Development

"Cooling was a landmark discovery for this disease," Higgins says. Finally, doctors (and their patients) weren't completely helpless. The intervention used in these studies reduced the number of newborns dying or enduring a severe disability to below 50 percent.

But to Higgins and other doctors, the improvements achieved with cooling were just the start. "It sounds wonderful that we've made significant improvement, but in my mind that's really just a proof of concept that you can make a difference," says Sandra Juul, the head of neonatology at the University of Washington School of Medicine and Seattle Children's Hospital. "But 50 percent is not acceptable."

Cooling sites of injury is an old concept, dating back to Hippocrates and Roman warriors, and practiced in modern times by any parent who's applied an ice pack to a boo-boo. Reducing temperatures slows cellular responses to damage, including cell death and subsequent inflammatory reactions. What cooling doesn't do, however, is heal damaged tissue.

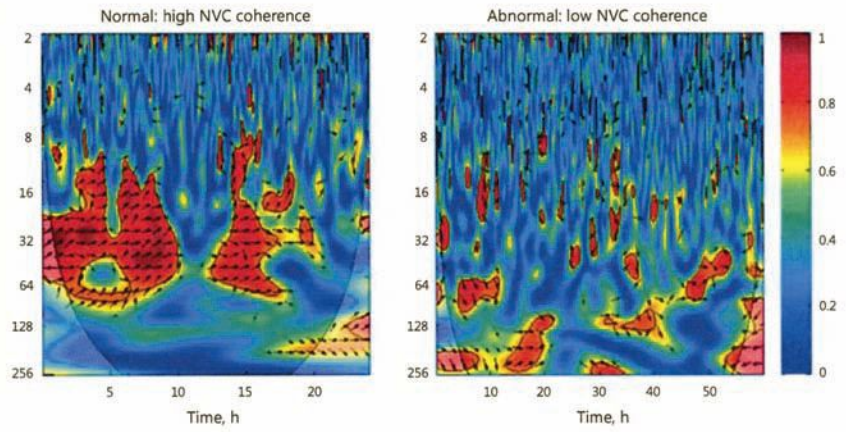
Investigators are therefore trying to boost the effects of cooling therapy and to find those babies who will benefit from extra interventions. There are some promising leads. Juul, for instance, is heading a phase 3 clinical trial to examine the effects of giving HIE babies erythropoietin, a hormone required for brain and red blood cell development, together with cooling therapy. Others are looking into adding xenon to babies' ventilators or administering antiseizure medications.

The challenge with any of these add-ons is that there is no good way to quickly assess how babies' brains are responding to treatment. As it is, there's no readily available technology that offers real-time reporting on progress in stopping brain damage. Doctors can use bedside assessments, such as whether the baby is conscious or has responsive pupils, and more high-tech exams, such as MRI. The former, however, isn't a precise method of predicting brain damage, and the latter is logistically challenging because the baby needs to be moved to a radiology suite.

Two more-practical diagnostic approaches are serum biomarkers of brain damage and physiologic recordings, such as electroencephalogram (EEG). Lina Chalak of the University of Texas Southwestern Medical Center in Dallas has worked on both. Most recently, her team designed a real-time "heat map" of babies' responses to cooling therapy that correlated with their developmental outcomes at 24 months of age.

Her team collected data from 10 newborns—eight of whom underwent cooling therapy, and another two who were normal and did not. For 60 hours they collected data from a method of EEG called amplitude-integrated EEG, which records trends in neural activity over time, and near-infrared spectroscopy (NIRS), which tracks blood flow and oxygenation. Combining the two metrics reveals so-called neurovascular coupling, Chalak says—essentially, how well neuronal behavioral and blood flow correspond. "You need neurons and blood vessels to communicate with each other."

Using a newly developed computational analysis, Chalak's group plotted this neurovascular coupling in real time on a color-coded heat map, reminiscent of the kind meteorologists use to display weather dynamics. Chalak's map revealed the synchrony within the babies' brains between their neural and vascular systems (*Scientific Reports*, 7:45958, 2017). Normal babies and babies with HIE who had better outcomes two years later showed higher neurovascular coupling (as indicated by big red islands in a sea of blue on the map). Babies without such patterning—showing instead a spattering of blue, yellow, orange, and red—tended



BRAIN STORMS: Looking like weather maps on the evening news, these graphs depict the amount of coherence between neuronal function and blood flow (NVC). An HIE baby that received cooling therapy showed greater coupling (left, red blobs), while a baby whose brain was damaged despite cooling therapy showed less (right).

to have poorer outcomes. "Neurons were firing, and blood vessels were not caring. There was no relationship," Chalak says.

She adds that the study is a proof of concept, and much more validation is required before the technique could earn a place at the bedside. Further along in validation studies are protein biomarkers present in the blood, which could be used to assess brain damage and how the brain is responding to treatment. Among them: proteins that signal astrocyte damage, such as GFAP (glial fibrillary acidic protein) and inflammatory cytokines. Although such biomarkers aren't collected continuously like EEG data, physicians could easily measure them from the blood draws that babies regularly endure throughout cooling therapy.

An ancillary goal of the erythropoietin trial is to assess the utility of these biomarkers in monitoring brain injury and tracking responses to the intervention. "I hope we're not too far away from a blood test for brain injury," says An Massaro, a neonatologist at the Main Hospital of the Children's National Health System, who is participating in the study. Chalak is also taking part, and will be adding her heat maps to the suite of data she collects on the infants. Massaro says she anticipates the study will provide answers not just on biomarkers and physiologic predictors of brain damage, but on ways to save more babies from the devastation of HIE. "I think it's going to be a very exciting time of clinical studies of brain therapeutics, but our big issue is identifying the right patients to put in these trials and the right patients to ultimately treat."

—Kerry Grens

SCIENTIFIC REPORTS, 7:45958, 2017

Why I Published in a Predatory Journal

Our totally bogus case report swiftly passed muster, with only minor revisions requested.

BY JOHN H. McCOOL

Earlier this year, I was invited to submit a paper to a dubious urology journal. I'm not a physician, much less a urologist. But I am an editor of scientific writing who has a strong antipathy for predatory journals. I'm also a *Seinfeld* fanatic.

So I decided to troll this publication, the MedCrave Group's *Urology & Nephrology Open Access Journal*, to see whether they would agree to publish a totally made-up, *Seinfeld*-themed "case report" about a man who develops "uromycitisis poisoning." This was inspired by the classic 1991 episode, "The Parking Garage," in which Jerry Seinfeld can't find his car in a mall lot, has to urinate, does so against a garage wall, is caught by a security guard, and tries to get out of a citation by claiming that he suffers from a condition called uromycitisis. Seinfeld argued that, due to his illness, he could die if he doesn't relieve himself when he feels the urge.

I went all out. I wrote my report as Dr. Martin van Nostrand, the physician-alter ego of another *Seinfeld* character, and listed more show-inspired names as bogus coauthors. I made an email account for Dr. van Nostrand and created a fake institution where the authors worked: the Arthur Vandelay Urological Research Institute. In the acknowledgments section of my report, I thanked phony physicians including Tor Eckman, the bizarre holistic healer from the Seinfeld episode "The Heart Attack," giving him a Doctor of Holistic Medicine (HMD) degree. Basically, I wrote the manuscript in a style as close to a real case report as I could, except that it was 100 percent fake.

To my surprise, a representative at *Urology & Nephrology Open Access Journal* wrote to say that my manuscript had been sent out for peer review. Three days later, it was conditionally accepted. I was asked to make minor revisions—including trimming the abstract and including the phony patient's lab results—and pay a "nominal" $799 fee, plus tax.

Continuing to dupe the publication, I did all that was asked—except remit payment—and, on March 31, my report was published on the journal's website. I have no intention of paying the requested fee.

A simple Google search for "uromycitisis" or "Martin van Nostrand" returns thousands of references to *Seinfeld*. Checking just one of the "papers" I included in the manuscript's reference section, the editors or reviewers could easily have determined it was fabricated.

Why did the journal publish a report so easily identifiable as fake? I'll leave that to the publication to explain.

Why, you might ask, did I take this stunt as far as I did? For nearly a year, I have been on a personal mini-crusade against fake scientific journals, and I have written several articles on the topic. In 2016, I was invited to submit a paper to the *Journal of Nanomedicine Research*, which is also published by MedCrave. I posted an article on LinkedIn about this, but it was not widely read, nor effective at exposing the journal as dubious. So when the urology journal came calling, I thought a more-extreme trolling operation might be more effective. I wrote the fictitious case report over a weekend.

My short-term goal is to expose MedCrave as a publisher that will print fiction, for a price. My long-term goal—an ambitious one, I know—is to stop the production of predatory journals altogether. ■

John H. McCool is the founder and senior scientific editor of Precision Scientific Editing, *based in Houston, Texas. A version of this article was published at the-scientist.com on April 6.*

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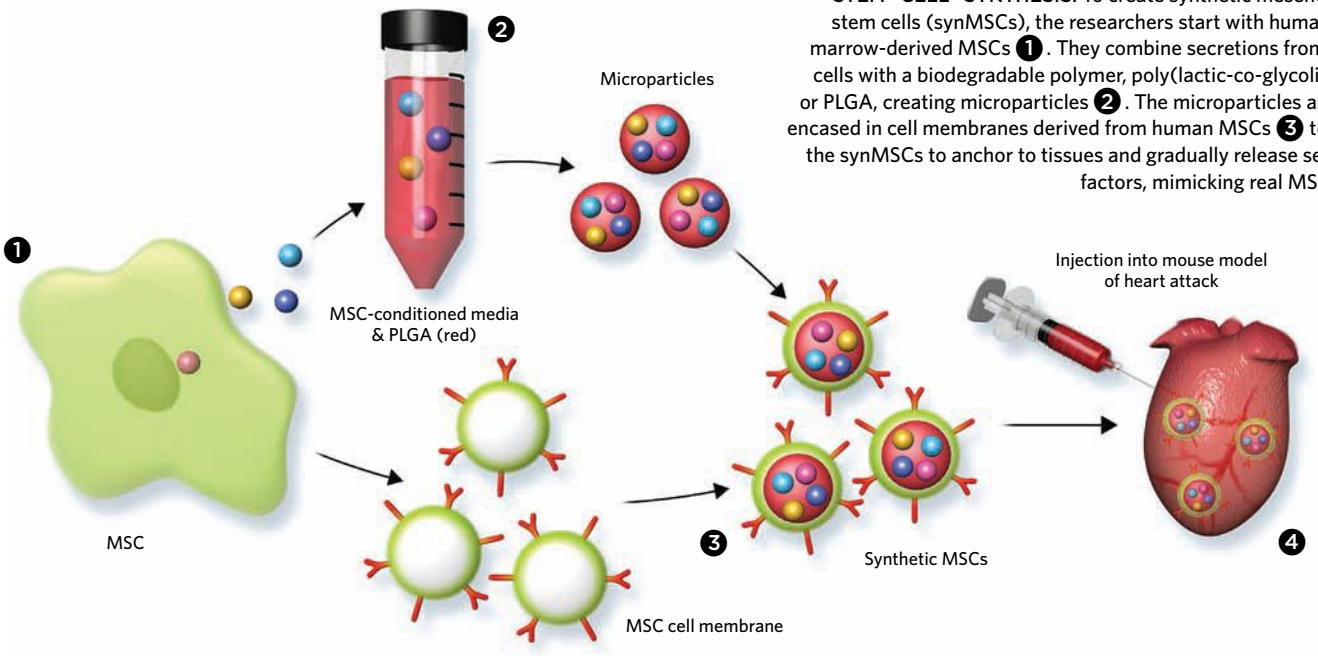
Synthetic Stem Cells

Engineered mesenchymal stem "cells" perform just as well as natural cells in regenerating mouse heart tissue.

BY DIANA KWON



STEM "CELL" SYNTHESIS: To create synthetic mesenchymal stem cells (synMSCs), the researchers start with human bone marrow-derived MSCs ❶. They combine secretions from these cells with a biodegradable polymer, poly(lactic-co-glycolic acid) or PLGA, creating microparticles ❷. The microparticles are then encased in cell membranes derived from human MSCs ❸ to allow the synMSCs to anchor to tissues and gradually release secreted factors, mimicking real MSCs ❹.

Mesenchymal stem cells (MSCs) are typically derived from adult bone marrow and fat tissue and are currently being tested in hundreds of clinical trials. They secrete proteins and other molecules that, when released to tissues, can promote regeneration, acting "like a pharmacy that provides drugs for tissues to heal," says Ke Cheng, a biomedical engineer at North Carolina State University and the University of North Carolina at Chapel Hill.

One limitation is that these cells need to be carefully frozen to keep them alive in storage, then defrosted, expanded, and gently maintained until used. "This process is tedious and sometimes can affect the potency of the cell," Cheng says. He also points out that some cells will inevitably die during handling, and injecting dying or dead cells into a patient can activate an inflammatory response.

To address these shortcomings, Cheng and colleagues engineered synthetic MSCs (synMSCs) built from human MSC secretions packaged in a biodegradable microparticle that was then coated with MSC cell membranes. These artificial cells withstood harsh cryopreservation and lyophilization (freeze-drying) without losing any of their properties. And when the researchers injected the membrane-bound particles into the hearts of a mouse model of myocardial infarction, the "cells" were able to promote regeneration to roughly the same degree as human MSCs.

SynMSCs could one day lead to off-the-shelf stem cell therapies, Cheng says—something that is very difficult to achieve with human- or animal-derived stem cells due to their fragility.

"I think it's a clever idea, and it has potential," says Joshua Hare, who develops cardiac cell therapies at the University of Miami Miller School of Medicine. "This is really a proof-of-principle demonstration, and there's a lot they have to do to get it to the level where it could actually be usefully applied." (*Circ Res,* doi:10.1161/CIRCRESAHA.116.310374, 2017) ■

AT A GLANCE

CELL TYPE	MODE OF DELIVERY	VIABILITY	APPROXIMATE SIZE	TESTED IN
Synthetic mesenchymal stem cells	Must be injected directly into site of action (e.g., heart)	At least one week at room temperature	20 μm	Mice
Mesenchymal stem cells derived from humans or other animals	Can be injected into blood vessels, because they will migrate to the site of injury	Around 24 hours at room temperature	20 μm	Humans, in multiple clinical trials

Running on Empty

Regularly taking breaks from eating—for hours or days—can trigger changes both expected, such as in metabolic dynamics and inflammation, and surprising, as in immune system function and cancer progression.

BY BOB GRANT

In 1971, a 27-year-old, 456-pound man went to the University of Dundee's department of medicine in Scotland looking for help. Patient A.B., as doctors referred to him, needed to lose weight. His physicians recommended a short but drastic course of action: stop eating altogether. The patient responded so well to a brief stint without food that he decided to prolong the deprivation—for more than a year.

"[H]is fast was continued into what is presently the longest recorded fast (*Guinness Book of Records*, 1971)," the clinicians wrote in a 1973 case report, claiming A.B. suffered little or no untoward effects on his health.[1] And at the end of his 382-day dietary abstinence, during which he had ingested only vitamin supplements, yeast, and noncaloric fluids, A.B. had lost a remarkable 276 pounds. When doctors checked back in on A.B. five years later, their patient reported gaining back only about 15 pounds.

Although aspects of this published report seem almost unbelievable, and the period of fasting is obviously extreme, the case highlights some of the metabolic dynamics that result when bodies are deprived of food. For example, when external calories stop fueling an animal's metabolism, stores of triglycerides in fat cells are mobilized, and levels of ketones—chemicals that result from the burning of fat for fuel—rise. Decreases in body weight follow.

Scientists are further detailing both the underlying metabolic dynamics and interesting physiological phenomena aside from weight loss as they study less-extreme permutations of fasting in animal models and in humans. Data have recently emerged from research on several forms of so-called intermittent-fasting regimens, including alternate-day fasting, the so-called 5:2 diet, time-restricted feeding, and periodic fasting (see definitions page 31). Although these regimens vary, they all involve a rhythmic disruption in the typical flow of calories into the metabolic machinery. "This is a simple intervention that has a profound impact," says Satchidananda Panda, a researcher at the Salk Institute for Biological Studies in La Jolla, California, who studies the effects of time-restricted feeding.

As the body of scientific literature around fasting has grown, results have been cherry-picked and molded into fad diets that promise weight loss, increased energy, better sleep, and a variety of other benefits to human adherents—some with more evidential backing than others. As books of dubious scientific merit extolling the virtues of fasting fill the shelves, serious researchers continue to probe the genetic, immunologic, and metabolic dynamics that occur in fasting animals to separate hype from reality.

Adapting to lean times

For the majority of genus *Homo*'s more than 2 million–year evolution, hominins' access to nutrients and calories was spotty, at best. Anyone who has hunted or gathered knows that success in either endeavor is not always guaranteed. But all of that began to change around 12,500 years ago, when *H. sapiens* invented agriculture, securing reasonably consistent streams of food—though the steadier diet constituted a narrower range of nutritional quality.

Perhaps our ancestors, and their digestive systems, evolved to endure periodic bouts of starvation, suggests University of Illinois at Chicago nutrition researcher

Krista Varady. "I'm positive we didn't have this constant flow or availability of food," she says of preagricultural times. "Really it was based on the hunt. . . . And between those times, we were just eating a lot of low-calorie foliage. Whenever the big hunt would come in with an animal, then we'd have food for a couple of days, and then we wouldn't."

These oscillations between feast and famine may even have served as a selective pressure, tuning early human physiology to function optimally in an environment where resources were unpredictable. "Individuals whose brains and bodies and physical performance were optimal in a fasted state would be more likely to get food and compete with other individuals who were not able to function at quite as good a level," says Mark Mattson, a neuroscientist who studies fasting diets at the National Institutes of Health's National Institute on Aging (NIA). "So the assumption then is that we evolved probably most of our organ systems to be able to function optimally in intermittent fasting–type conditions."

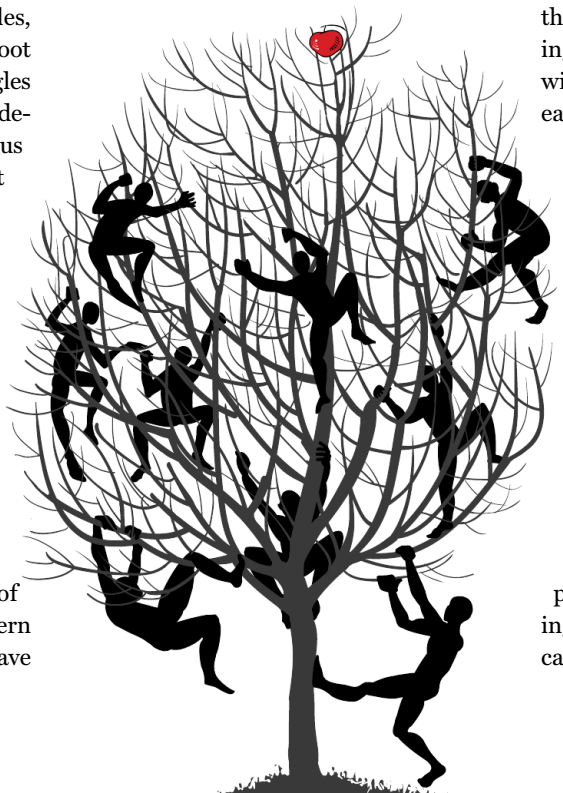
Panda, however, downplays the importance of feast and famine cycles in shaping our ancestors' evolution. Instead, he contends that more-contemporary disruptions to the body's many circadian clocks—which sense dark/light and feast/famine cycles, among other oscillations—are at the root of modern humanity's mounting struggles with obesity and metabolic disease. "Undeniably, the amount of food available to us has increased, but another thing that has happened is the invention of [electric] light," he says. "When there was darkness in the evening, of course people didn't have much to do. . . . The light enables us to stay awake later in the night. And now we have plenty of food, so we tend to eat."

Even with this disagreement concerning evolutionary vs. industrial justification for designing modern-day fasting diets, results from studies in both animal models and humans point to distinct benefits of withholding food in one temporal pattern or another. In recent years, scientists have learned that fasting might trigger not only weight loss and life-span extension—benefits that have long been linked to caloric restriction—but also boost the performance of the brain, the immune system, and organs central to metabolism, such as the liver and pancreas. Fasting, some researchers claim, can even alter the course of some diseases, from cancer and multiple sclerosis to diabetes and Alzheimer's.

The fasting signal starts

In seeking to understand how a body reacts to a dearth of incoming calories, it makes sense to start in the liver, an organ crucial for the processing of nutrients flowing into a body's metabolic machinery. Panda has documented profound alterations in gene expression in the liver cells of animals subjected to conditions of fasting. In a 2009 *PNAS* study, he and colleagues found that when they withheld food from mice for 24 hours, 90 percent of the genes expressed in a cyclical, circadian clock–driven fashion by liver cells ceased to oscillate.[2] "That means most of the cycling comes from these instructions from food," he says.

Oscillations between feast and famine may have served as a selective pressure, tuning early human physiology to function optimally in an environment where resources were unpredictable.



Conversely, Panda's lab later showed that feeding mice high-fat diets with food accessible 24 hours a day similarly breaks circadian clocks in the liver, resulting in an enhanced propensity for obesity and its attendant maladies. But making that same high-fat diet—and the same number of calories—accessible to the animals for only eight hours per day during their normal wake cycles protected the mice from the development of a host of metabolic diseases.[3] "The mice were completely protected from diabetes, cardiovascular disease, high cholesterol, fatty liver disease, these diseases that affect most of our elderly," he says. (See "Out of Sync," *The Scientist*, September 2013.)

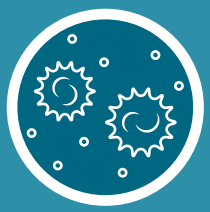
Altering the feeding window to regimens of 8, 9, or 12 hours didn't make much of a difference. "In most cases we saw protection from all of these diseases, and if the mice were fat enough initially, they lost 20 percent of their body weight and many of their diseases reversed," Panda says, adding that they also tried a 15-hour feeding window, which did not offer too much disease protection.

Panda says that the mechanisms underlying the physiological responses to fasting likely involve a number of metabolic pathways commonly studied by nutrition scientists, including mTOR, insulin, AMP kinase, and protein kinase A (PKA). These same pathways turned up recently in a study by the University of Southern California's Valter Longo, who examined the consequences of fasting on another crucial metabolic organ, the pancreas. He and collaborators showed that periodic fasting using a fasting-mimicking diet (FMD)—which is low in calories, carbohydrates, and proteins but contains

A BODY WITHOUT FOOD

Mounting evidence suggests that intermittent fasting causes significant changes to various organs and tissue types. The fasting signal likely starts in the liver, the body's central command for metabolism. But through changes in gene expression and alterations in complex enzymatic pathways, the effects of food deprivation spread throughout the body, from the brain and visceral fat to the muscles and more.

LIVER
Fasting and time-restricted feeding increases insulin sensitivity, decreases insulin resistance, and lowers blood glucose levels. With prolonged periods of fasting, the liver's glycogen stores become depleted, and visceral fat is tapped as an energy source, which releases ketones that can be metabolized by neurons and muscle cells.



IMMUNE SYSTEM
Periodic fasting reprograms T-cell populations, tamping down autoimmunity and rescuing immunosenescence. A lack of incoming calories appears to prune away autoimmune T cells, and with refeeding, hematopoietic stem cells are activated to replace T cells, lymphocytes, and other white blood cells. Several fasting studies have also pointed to a decrease in inflammatory cytokines.

HEART
Because triglycerides become mobilized for energy in the absence of incoming dietary calories, blood lipid levels tend to go down in a fasting body. Researchers have also seen decreases in blood pressure in fasting animals. In some animal studies of fasting, investigators have recorded decreases in cholesterol.

BRAIN
Intermittent fasting has improved memory, learning, and neurogenesis in rodents, and has been shown to repair some neurons in mouse models of ischemic stroke.

CANCER
By making tumor cells more susceptible to chemotherapeutic agents while protecting healthy cells from the treatment's toxicity, intermittent fasting is showing promise in slowing the progression of breast cancers and melanoma in mice.

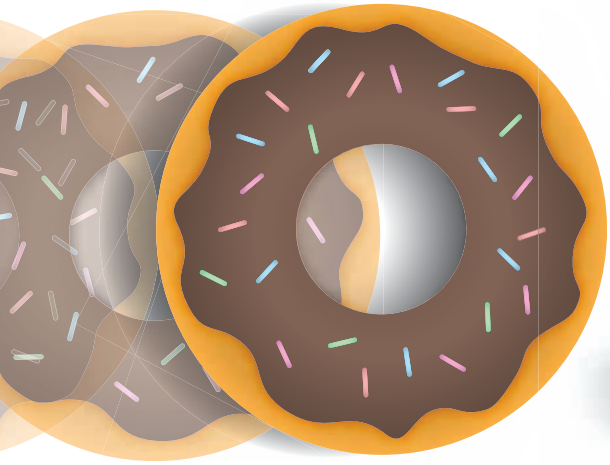
ample fatty acids to push the body into a fasting state without a complete cessation of incoming energy—promoted the functional repair of damaged pancreatic cells in mouse models of type 1 and type 2 diabetes by reducing PKA and mTOR activity, while increasing insulin production.[4] (Longo started a company, L-Nutra, which sells FMD diets; he donates his profits to charity.)

The hungry brain

Mattson, who studies the brains of mice, rats, and humans in his lab at the NIA, has identified neurological benefits of fasting and suggests that the adaptive stress response may be at their foundation. The gist of this response is that, under unfavorable environmental conditions such as toxin exposure, extreme heat, or a lack of readily available fuel, cells in an animal's body alter gene expression to crank out proteins that play protective roles. These proteins make cells more resistant to the stressor through enhancing DNA repair or by stabilizing proteins' 3-D conformations, among other mechanisms. In the brain, the adaptive stress response can strengthen neuronal networks and enhance neural plasticity.

In recent years, Mattson and colleagues have homed in on brain-derived neurotrophic factor (BDNF) as a key mediator of neurological and other fasting responses. Their research has revealed that this growth factor causes an increase in the number of mitochondria in hippocampal neurons, perhaps explaining why rats on fasting cycles not only gain less weight than their continuously fed counterparts, but perform better at learning and memory challenges.[5] Intermittent fasting appears to calm inflammation and rescue cognitive function in the animals' brains by knocking down the quantity of proinflammatory cytokines, elevating levels of BDNF, and activating the transcription factor known as NF-κB, which is involved in adaptive stress responses.[6] In addition, BDNF activates PGC-1α, which regulates the production of new mitochondria, dendritic spines, and synapses in the hippocampus.[7]

Last year, Mattson and collaborators further elucidated the complex molecular mechanics that link intermittent fasting with improved brain function and neuroprotection. The researchers demonstrated that the mitochondrial deacetylase SIRT3 is central to mediating adaptive stress responses in mice that are exposed to prolonged exercise or periods of fasting. Under these conditions, SIRT3 levels in hippocampal neurons ramp up, improving neural network function and boosting mitochondria production in the hippocampus.[8] "There's a story emerging whereby exercise . . . and intermittent fasting as well, upregulates SIRT3 and protects mitochondria against stress," says Mattson. "And that seems important for exercise and intermittent fasting to allow the cells to grow and new synapses to form."



Rebuilding defenses

Fasting that involves longer periods of food deprivation can cause changes to the immune system and the hematopoietic stem cells that support it. Longo studies periodic fasting, less frequent but longer bouts of severe calorie restriction, and is finding that the routine can reshape immune cell populations in the body. For most of his studies, Longo uses the fasting-mimicking diet (FMD).

Using a periodic three-day FMD regimen for 30 days in a mouse model of multiple sclerosis, Longo and colleagues showed that the fast-and-feed cycles pruned away populations of autoimmune T cells, replacing them with immune cells that were no longer bent on attacking neural tissue. Oligodendrocyte precursor cells regenerated and remyelinated axons, and the clinical severity of the autoimmune disorder declined.[9] "One in five of the mice went to back to no symptoms at all. One in two of the mice went down to very low levels of the symptoms," Longo says.

Fasting that involves longer periods of food deprivation can cause changes to the immune system and the hematopoietic stem cells that support it.

"The real benefit that we've shown in a number of papers, and we have several coming out very soon, is about killing damaged cells and then turning on stem cells," Longo adds. "And then in the refeeding period, [stem cells are] replacing the dead cells with newly generated cells. I think that is where the real benefit is."

Healing, fast

Much of the basic biological work on fasting diets is done in animal models of disease, demonstrating improvements in or protection against symptoms of Alzheimer's, multiple sclerosis, cardiovascular disease, diabetes, and other metabolic disorders. Longo has also turned his sights on cancer. He and his team showed in mouse models of breast cancer and melanoma that periodic fasting with a four-day FMD for about a month, combined with chemotherapy, delayed tumor progression by sensitizing the malignant cells to chemotherapy, while protecting normal cells from the toxins. This effect, they found, was mainly mediated by increases in the levels of lymphoid progenitor cells and tumor-killing lymphocytes.[10] "On one side, the fasting promotes the regeneration of the immune cells and they're more aggressive, but more importantly, it's acting on the breast cancer cells and also the melanoma cells to make them more exposed in a way to the T cells," Longo says.

Mattson, too, has tested the effects of fasting on specific disorders. In 2014, he participated in research that illustrated the contribution of time-restricted feeding—in this case, feeding mice in an eight-hour window every day for four months—to tamping down inflammatory responses after ischemic stroke was induced in the animals. The study implicated some of the same neuronal signaling pathways—NF-κB and MAPK, for example—in the fasting regimen's ability to calm the cytokine storm that attacks brain tissue after stroke.[11] "There's some evidence coming out that intermittent fasting after stroke in animals can enhance their recovery," Mattson says.

Longo, working as the director of USC's Longevity Institute, is now directing his questions about fasting and cancer progression to humans. "Now we have about 400 patients that are enrolled or will be enrolled in multiple clinical trials that we're running with the fasting-mimicking diet," he says.

The fasting fad

As often happens with nutrition research, results from fasting research have been borrowed and packaged into ill-informed fad diets. Panda's research formed the basis of one such product, branded as

The 8-Hour Diet. The book, cowritten by an editor at *Men's Health,* which covered Panda's research when it came out in 2012, claimed (right on the cover) that dieters who restricted their food consumption to an eight-hour window every day could "watch the pounds disappear without watching what you eat!" This idea, says Panda—who was not involved in producing the book—was unsupported by his research as of 2012. "We never claimed that we could make fat mice lean with the eight-hour diet," he says. "In our first paper, we just prevented obesity from happening."

Nevertheless, Panda has gone on to show that there may be some substance to the weight-loss hype. In addition to his experiments in mice, Panda's team found that flies, too, experienced benefits from restricting their feeding windows to a confined period each day. For the insects, a 12-hour feeding window proved to be best, with flies eating for only half a day experiencing better-quality sleep, less weight gain, and a deceleration of cardiac aging compared to their counterparts who were fed the same number of calories, but in food administered throughout the day.[12]

Extending the research into humans, Panda's lab recently published a study of eight otherwise healthy people with body mass indices between 25 and 30 (in the "overweight" range, according to the National Health and Nutrition Examination Survey). When the study subjects ate all of their food for the day in 10 hours, instead of 14 or 15 (as they tended to do before the study), they lost an average of 4 percent of their body weight and reported having significantly more energy and more satisfying sleep over the 16-week trial period, with the benefits sticking around for up to a year.[13]

Most intermittent-fasting regimens, including alternate-day fasting and the 5:2 diet (fasting for two days each week), have shown promise in helping people and experimental animals shed pounds. Unlike time-restricted feeding, however, these approaches are really caloric restriction in disguise. By severely limiting calories every other day or every third day, dieters reduce their caloric intake over the course of a week or month, without the annoyance of constantly counting calories or restricting portions at every single meal. "That's what intermittent fasting is, it's just tricking your mind and body into eating less, and because you're losing weight, you're getting all these metabolic benefits," says Varady, who studies alternate-day fasting and wrote a book, *The Every-Other-Day Diet,* as a summary of her research and a recipe guide. Last month, she and colleagues published results of a clinical trial comparing obese, but otherwise healthy, people practicing alternate-day fasting to a similar group restricting calories on a daily basis. Both cohorts lost roughly the same amount of weight over the course of one year, and both diet regimes had similar noncompliance rates.[14]

Varady notes that most of the trial subjects on whom she's tested alternate-day fasting tend to report a certain amount of ease with complying with the diet, and she's found that they tend not to compensate for fasting days by consuming drastically more calories on feast days. "It's almost like they can't binge on the feast day. They typically only consume about 15 percent more energy than they normally do," she says.

Richard Bloomer, a University of Memphis nutritional scientist who studies dietary restriction in animals and humans, says that long-term compliance is still an unanswered question about the practicality of intermittent-fasting diets. "The compliance issue is crucially important. Which of these plans, regardless of the outcomes, allow people to maintain them longer term?" he asks. "That's probably one of the more important things to study as opposed to simply the mechanisms as to why things are occurring."

And for some researchers, intermittent-fasting plans, such as alternate-day fasting and the 5:2 diet, are doomed to a fate similar to that of caloric restriction. "Intermittent fasting, whether you do two days a week, every-other-day fasting—some of the things that are being used the most—they're extremely difficult for people to do," says Longo. "There's very little data, actually, long-term, and my guess is that, just

TYPES OF INTERMITTENT FASTING

All fasts are not created equal. Researchers are studying several different permutations of intermittent fasting, which all provide ample drinking water to subjects, in laboratory animals and in humans.

Alternate-day fasting: Subjects eat every other day. For humans, noneating days typically consist of one small meal of around 500 calories, amounting to a dietary energy reduction of approximately 65 percent to 80 percent.

5:2 diet: A person eats five days of the week and abstains from eating the other two (for example eating on Monday, Wednesday, Friday, Saturday, and Sunday and fasting on Tuesday and Thursday)—save for one small, 500-calorie meal on fasting days (cutting dietary energy by about 65 percent to 80 percent on those days).

Periodic fasting: This fast is undertaken anywhere from once a month to once a year. For a period of at least five days, food is avoided or subjects eat a modified "fasting-mimicking diet" that steps down energy intake over the fasting period and is low in carbohydrates, proteins, and calories.

Time-restricted feeding: Calories are not restricted, and dietary composition is not altered. But eating is confined to a window of typically 8, 10, or 12 hours per day.

SELF-EXPERIMENTERS

In the tradition of self-experimentation, many of the researchers who study intermittent fasting follow the plan to which they subject laboratory animals or human subjects. Here are the diets of a few of the leading fasting scientists:

Mark Mattson, NIA
Eats within a six-hour window every day and does trail running

Valter Longo, USC
Eats twice per day (skipping lunch) and practices a periodic fast for five days every six months

Satchidananda Panda, Salk Institute
Eats within a 12-hour window every day and practices an extended water-only fast of five days once per year

Krista Varady, University of Illinois at Chicago
Practices alternate-day fasting one or two months per year, "usually after Christmas to shed the five pounds of holiday weight."

like caloric restriction, you're going to see the good and the bad, and it's potentially going to be a failed operation."

"I think intermittent fasting right now is probably another nutritional fad," says Varady. "I've been in nutrition research not for a super long time but for about 15 years or so, and I just noticed every 10 or 15 years there's something new that comes up."

Nevertheless, she adds, "I still think that it can really help people out, and I think people who are able to stick to it really reap a lot of metabolic benefits." ■

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Intermittent fasting, whether you do two days a week, every-other-day fasting—some of the things that are being used the most—they're extremely difficult for people to do.

—Valter Longo
University of Southern California

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The Celiac Surge

A rapid increase in the global incidence of the condition has researchers scrambling to understand the causes of the trend, and cope with the consequences.

BY CATHERINE OFFORD

In 2015, gastroenterologist Edwin Liu set to work on a clinical and genetic data set that had been growing for more than 20 years. The data pertained to celiac disease, a lifelong condition involving bouts of severe gastrointestinal distress and other symptoms, triggered by ingestion of gluten proteins that are found in wheat and several other grains. In a two-decade collaboration with researchers at Children's Hospital Colorado in Denver, Liu's predecessors and colleagues at the University of Colorado kept track of 1,339 babies born in the city who were deemed at risk of developing the disease due to mutations in celiac-linked genes. The researchers carried out yearly tests to see whether or not the children developed the disease, hoping to better define the risk associated with each of the genetic variants.

Not far into his analyses, however, Liu found something in the data that undermined a much larger assumption in the celiac field. "Usually, when we quote numbers for celiac disease, we're quoting around 1 percent" prevalence in the US population, he says. But using data from this cohort along with estimated frequencies of each genotype across the Denver metro area to extrapolate the incidence of celiac disease to the general population, Liu found that the true prevalence of celiac disease had to be much greater—more than 3 percent by age 15. "It was a surprise," he says. "These numbers are much higher than anything else quoted in the U.S."

Researchers reading the paper, which was published online earlier this year in *Gastroenterology*,[1] were similarly taken aback. "If you look at the rates, it's frightening," says Joseph Murray, a celiac researcher at the Mayo Clinic in Rochester, Minnesota. Of course, the statistic could be specific to the Denver cohort, he notes, but it does fit in with similar trends reported both in the U.S. and around the world.

Celiac symptoms, which include abdominal pain and distension, diarrhea and flatulence, nausea, and fatigue, are brought on by ingestion of gluten—a protein complex present in wheat, barley, and rye. Unlike food allergies, which are often primarily mediated by an overreaction of adaptive immune responses such as immunoglobulin E antibody production and mast cell activation, celiac disease engages both innate and adaptive immune pathways, and produces antibodies that target not only gluten, but the body's own proteins. As a result, the disease is generally considered an autoimmune condition. (See illustration on page 36.) Triggered by even tiny amounts of gluten, these immunological attacks lead to T cell–mediated atrophy of the gut wall, which can be characterized via a biopsy of the small intestine for celiac diagnosis (see "Diagnosing Celiac Disease" on page 37).

As the use of biopsy and other diagnostic methods have improved in recent decades, celiac disease has become easier to detect. So when the first reports of increasing numbers of celiac cases in the U.S. came out in the early 2000s, many researchers attributed the uptick to progress in disease recognition. But closer scrutiny of the data suggested there was more going on. "We weren't just better at finding celiac disease," Murray says. "There was a lot more of it to go around."

By comparing blood samples taken from young adults in the Air Force around 1950 with matched samples from residents of a Minnesota county collected since 1995, for example, Murray's group estimated an increase in prevalence from 0.2 percent to nearly 1 percent.[2] Sweden, meanwhile, experienced what is now referred to as a celiac epidemic in the late 1980s and early 1990s, with one study estimating that as many as 3 percent of children born at the height of the epidemic had developed celiac disease by the age of 12—though rates dropped back down to just over 2 percent for children born in 1997.[3] And several studies based on blood tests suggest increasing numbers of people are developing celiac disease in wheat-eating areas of northern India, with a prevalence in children of around 1 percent and some researchers warning of an impending epidemic there too.

AROUND 40 PERCENT OF PEOPLE HAVE THE GENES PREDISPOSING THEM TO CELIAC DISEASE. THE BIG QUESTION IS WHY SOME PEOPLE GET IT AND OTHERS DON'T.

—Edwin Liu, University of Colorado

The cause of this apparently global trend remains a mystery, not least because, while the immunopathology of celiac disease has been studied for decades, just what causes people to develop the ailment in the first place remains unclear. Almost all diagnosed patients have mutations in at least one of the two genes coding for HLA-DQ, a membrane receptor on antigen-presenting cells that helps the immune system distinguish self from non-self and coordinate T-cell activity. But not everyone who has such risk genes gets celiac. "Around 40 percent of people have the genes predisposing them to celiac disease," Liu explains. "The big question is why some people get it and others don't." Hypotheses abound, with many pointing the finger at a gluten-rich diet, but evidence to support these ideas remains far from conclusive.

Getting to the bottom of this question will be necessary not only to curb the concerning trend, but also to help doctors

better detect and manage the multifarious disease, for which the only current treatment is a gluten-free diet. In addition to celiac's sometimes-debilitating symptoms, the disease is associated with a heightened risk for numerous conditions, including autoimmune diseases such as diabetes and hypothyroidism, and myriad other disorders, from infertility to small-bowel cancer. Overall, celiac patients have up to a twofold increased mortality risk compared with the general population.

"The stakes are high," says Murray. "If this disease has gone from being a truly rare disease in some geographies to being a common disease affecting 2 or 3 percent of children, that's no longer a small disorder."

Why the rise?

One thing celiac researchers agree on is that the direct cause of the rise in the disease likely resides outside of our DNA. "Over decades, it's just too quick for genetic changes to occur," Liu says. "We have to assume that this is based on environmental factors." There's still little in the way of concrete answers as to what these factors might be, however. "I've heard every type of hypothesis that's been thrown out there," says Murray, "but most are them are not easily testable."

Some of the more unusual candidates blamed for triggering celiac disease include microwaves, plasticware, and diatomaceous earth—an abrasive powder applied to flour containers as an insecticide—although scientific evidence to incriminate these supposed culprits is scant. Other factors that have more reliably been tied to increased celiac risk in genetically predisposed infants include delivery by Caesarian section, and intestinal infections by pathogens such as reovirus (recently implicated in a mouse model)[4]—although their impacts are likely minor, Murray says.

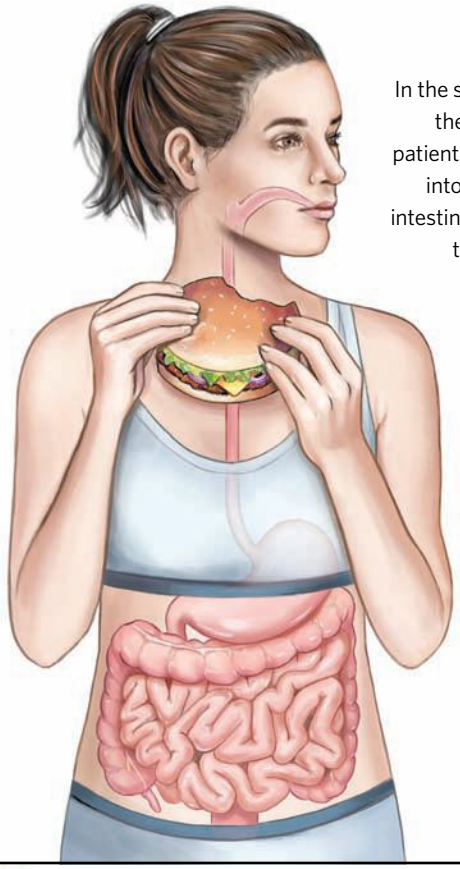
The role of gluten itself—the immediate trigger for the immune responses in celiac patients and therefore, researchers have long assumed, a crucial player in the epidemiology of the disease—has also remained frustratingly elusive. During the 2000s, for example, several observational studies pointed to a suite of dietary factors, including age of gluten introduction, as influencing the development of celiac disease. But the findings suffered a blow in 2014 when two randomized clinical trials failed to find any effect of the timing of gluten introduction.[5,6] The studies also found no evidence for a link with the duration of breast-feeding—a factor that had previously been touted as protective against developing celiac disease. Gastroenterologist Alessio Fasano of Massachusetts General Hospital, a coauthor on one of the publications, says researchers realized then that "the story is much more complex than we thought."

A more recent hypothesis is that the amount of gluten consumed, if not the timing, could play a role in triggering celiac disease in children. The US Food and Drug Administration (FDA) notes that wheat consumption increased rapidly in the second part of the 20th century as people began to eat less meat and consume increasing amounts of readily available, wheat-containing fast foods. (A more controversial idea is that the composition of wheat has changed significantly during this time—search "Frankenwheat.") And rising incidence of celiac disease in South Asia tracks with the widening adoption of Westernized diets, although data on gluten consumption per se is lacking.

Some evidence that these dietary changes could be tied to the rise in celiac disease comes from a retrospective 2016 study of Swedish infants, which suggested that genetically susceptible children consuming more than 5 grams of gluten per day—the equivalent of about one slice of whole wheat bread—before 2 years of age were up to two times more likely to develop celiac disease than those consuming less than that amount.[7] "[The result] tells me that the amount of gluten matters," says Murray. "I think we have to go back and revisit what's happening with gluten—how much are we eating, and is it a potential risk factor?"

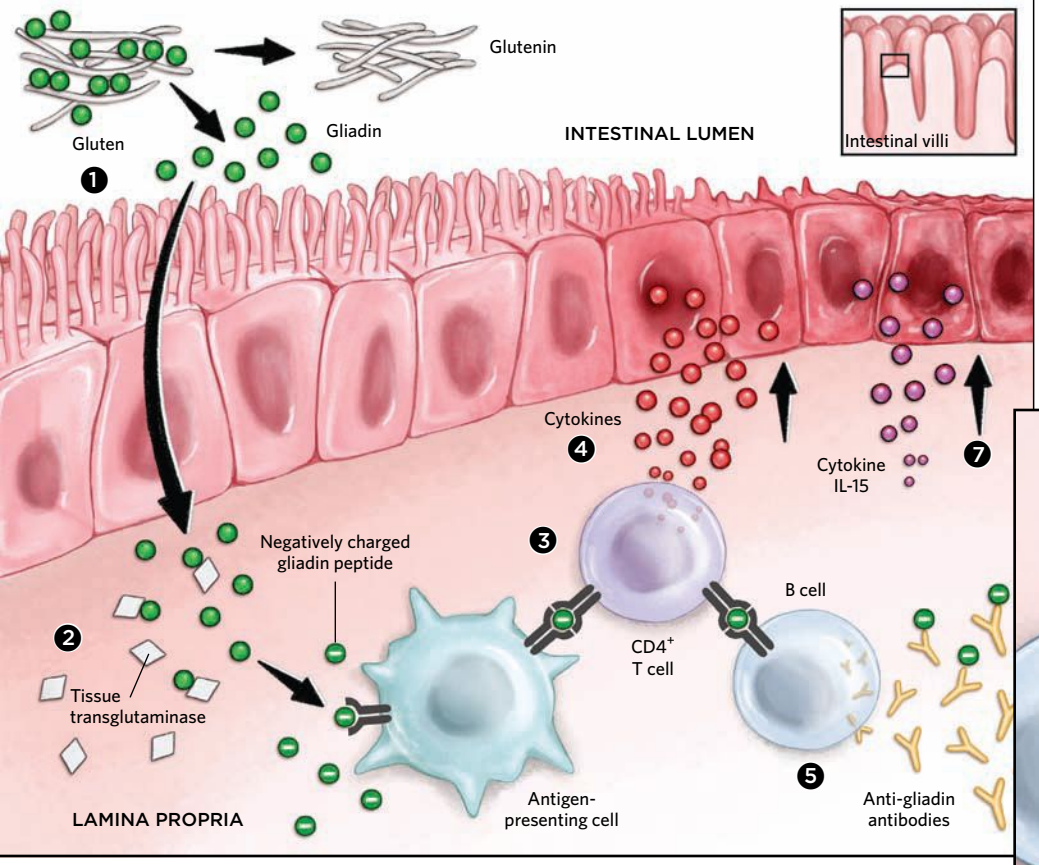
Reactions to these findings have been mixed, however. "The evidence was fairly weak," notes celiac researcher Detlef Schuppan of Johannes Gutenberg University Mainz in Germany. In terms of the global rise in celiac prevalence, "the amount of gluten ingested does not explain it," he adds.

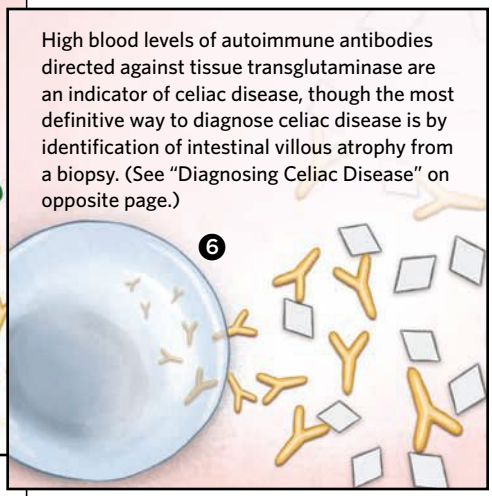
Amidst uncertainty about gluten's part in the celiac trend, many researchers are quick to point out that it's not just our diet that has changed in the last century. One factor now under scrutiny across digestive diseases and beyond is humans' usage of antibiotics and, consequently, the composition of bacteria making up the gut microbiome. (See "The Sum of Our Parts," *The Scientist,* July 2015.) Bacteria living in the gut play important roles in metabolism and in the regulation of immune responses to food, so for many researchers, these microbes are likely suspects in celiac disease pathogenesis. According to this line of thinking, "maybe the bugs we've now got are not as happy when they interact with gluten," Murray says. "Or the results are not as good for us when these bugs interact with gluten as when our old bugs did."



IMMUNE IRRITATION IN THE GUT

In the small intestine, gluten proteins are broken down into their component glutenins and prolamins ❶. In celiac patients, the prolamin component of wheat, gliadin, passes into the lamina propria, a layer of tissue underneath the intestinal epithelium, and is processed by an enzyme called tissue transglutaminase ❷. The resulting negatively charged gliadin peptides are picked up by antigen-presenting cells that display them for assessment by the immune system ❸. When $CD4^+$ T cells recognize gliadin, they release cytokines that trigger inflammation ❹ and stimulate B cells to produce antibodies that target gliadin ❺ as well as autoimmune antibodies that target tissue transglutaminase ❻. In addition, gliadin triggers upregulation of the cytokine IL-15 in the lamina propria and gut epithelium, prompting further inflammation ❼. Together, these responses cause considerable atrophy of the intestinal villi—projections of the small intestinal wall that facilitate efficient absorption of nutrients. This atrophy can lead to long-term dietary deficiencies and increased risk of other disorders such as lymphoma.





High blood levels of autoimmune antibodies directed against tissue transglutaminase are an indicator of celiac disease, though the most definitive way to diagnose celiac disease is by identification of intestinal villous atrophy from a biopsy. (See "Diagnosing Celiac Disease" on opposite page.)

The last two decades have seen a number of observational studies report abnormal microbiome composition in the guts of celiac patients compared with healthy controls. Patients with celiac disease show a higher proportion of gram-negative bacteria such as *Bacteroides* and *E. coli*, for example, and some evidence suggests that those displaying gastrointestinal symptoms also have higher levels of Proteobacteria. Another bacterium, *Helicobacter pylori*, has been associated with protection from celiac disease, and declines in the number of adults carrying this microbe in their guts appear to have coincided with increases in the number of celiac cases in the U.S.—although research on this subject remains inconclusive.

Whether these differences in microbiome composition are the cause or the consequence of celiac disease remains unanswered, says Bana Jabri, director of research at the University of Chicago Celiac Disease Center. "You could think about it in several ways," she explains. "Maybe there's a difference in the microbiota from the beginning, and this has a causative role. Or maybe what you're seeing is just a secondary effect. At this point, we really don't know."

Nevertheless, circumstantial evidence is accumulating to suggest more than a passive role for these microbes. For starters, it's known that changes in the microbiota can induce different types of immune responses, and celiac patients often continue to show abnormal gut

flora even after adopting a gluten-free diet. Additionally, Jabri's group showed last year that mice engineered to overexpress interleukin-15—a cytokine involved in celiac disease pathogenesis—had restructured microbiota as well as altered production of certain fatty acids, mirroring precursors of intestinal inflammatory diseases in humans.[8] "When you put all this together, you could say that there really is enough evidence to believe in a causative role for the microbiota," says Jabri. "But the critical experiments still need to be done."

With so many factors being investigated, Liu says, it's unlikely the explanation for an increase in celiac disease incidence will be simple. "I don't think we're going to be able to find a single environmental trigger," he says. "It's going to be a combination." Murray takes a similar view. "There are so many things going on, so many moving parts," he says. "The challenge for us as scientists is to reduce it down to testable hypotheses."

Damage control

While scientists grapple with how to explain the disease's underlying causes, the rise in celiac prevalence is prioritizing the condition in the medical community and highlighting the need for improved diagnosis and management of existing cases. It's worth remembering, says Murray, that celiac disease is a lifelong condition. "When you develop celiac disease, you can't undevelop it," he says. "You can heal it, by avoiding gluten, but you can't put Humpty Dumpty together again. The immune system has changed."

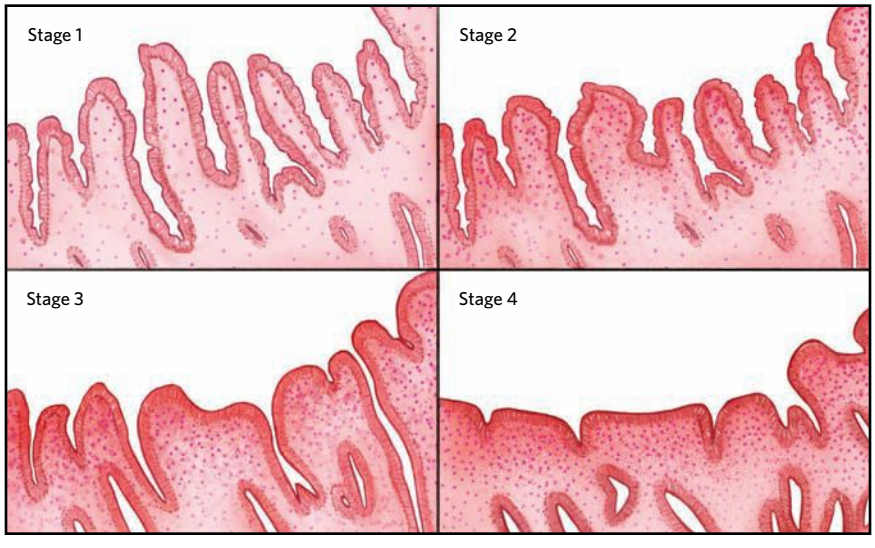
For now, the only treatment is a gluten-free diet. But as the focus on celiac disease has intensified, so too has research on the effects of this intervention—and the results are not encouraging. Several studies suggest that celiac patients' guts are unlikely to heal completely even on such a diet, and accidental ingestion of the ubiquitous protein is almost inevitable; even tiny amounts can trigger symptoms. Moreover, patients find the gluten-free regimen difficult to tolerate because it is isolating and often impractical. In 2011, University of Sheffield celiac researcher David Sanders and his team surveyed 310 people diagnosed with celiac disease who were following a gluten-free diet, and found that more than 40 percent were dissatisfied.[9] "Most people eat three times a day, and with celiac disease, that's a challenge," says Sanders. "Every single day, this problem is right in front of [them]."

> WHEN YOU DEVELOP CELIAC DISEASE, YOU CAN'T UNDEVELOP IT. YOU CAN HEAL IT, BY AVOIDING GLUTEN, BUT YOU CAN'T PUT HUMPTY DUMPTY TOGETHER AGAIN. THE IMMUNE SYSTEM HAS CHANGED.
>
> —Joseph Murray, Mayo Clinic in Rochester, Minnesota

To improve outcomes for patients with celiac disease, some scientists are exploring ways to tackle the celiac gut's response to gluten and potentially restore tolerance. Celiac researcher and chief scientific officer of ImmusanT, Bob Anderson, for example, is expanding work he began during a postdoc at the University of Oxford to develop a vaccine that could help desensitize patients to gluten over a series of injections. The vaccine, Nexvax2, comprises three peptides—components of gluten taken from wheat, barley, and rye—that trigger an adaptive immune

DIAGNOSING CELIAC DISEASE

A blood test can be used to screen for the presence of tissue transglutaminase antibodies, which can indicate celiac disease, but a diagnosis is only confirmed with an intestinal biopsy. Doctors take samples of the small intestine lining and quantify villous atrophy on a scale known as the Marsh Score from 0 to 4, where 0 represents normal villi covered with invaginations, and 1 through 4 denote increasing levels of atrophy. Stages 1 and 2 represent possible celiac disease, stage 3 signifies symptomatic celiac disease, and stage 4 reflects total atrophy.



response in celiac patients. Phase 1 results showed the vaccine to be safe, and Phase 2 trials are planned for later this year, Anderson says. "It's a highly targeted approach to try to engage the bulk of the gluten-reactive T cells that play a pivotal role in causing and maintaining the disease," he explains.

There are less orthodox approaches in the works, too. In recent years, researchers in Australia have been investigating the idea that attacking the body with intestinal parasites could help mitigate the effects of autoimmune disease by "giving the immune system something to do," says Sanders, who was not involved in the work. In 2014, the team reported results from 12 people with celiac disease who, after being experimentally infected with hookworm larvae—which then migrated to the gut and grew—could follow a normal diet with significantly reduced symptoms.[10] The same researchers plan to launch a follow-up, double-blind randomized trial with 60 participants later this year, and have stated that the results could inform the future development of new, non-parasite–based therapies.

Other experimental therapeutics for celiac disease would require patients to continue a gluten-free diet, but serve to reduce the severity of reactions to accidental exposure when taken before a meal. Baltimore-based pharmaceutical company Alba Therapeutics—cofounded by Fasano—has developed pills containing a synthetic peptide known as larazotide acetate, which inhibits a protein called zonulin that regulates epithelial-cell tight junctions. Zonulin is thought to increase the permeability of the gut, and is upregulated in people with celiac disease—factors that company researchers suggested could explain why the drug reduces gastrointestinal symptoms in celiac patients following gluten exposure. The therapy was licensed out for Phase 3 trials last year. Another experimental drug, developed by California-based Alvine Therapeutics and recently acquired by ImmunogenX, contains a mixture of two gluten-targeting proteases—one plant-derived and one derived from bacteria. The drug chops gluten molecules into successively smaller pieces, and reduced gut tissue damage in celiac patients who ingested small amounts of the protein in a Phase 2 trial.

With these alternative treatments a long way from being available for general use, many health-care providers argue that improved diagnosis should be the priority, to help better understand and care for the increasing numbers of people living with celiac disease. In 2014, a team at the University of Nottingham reported significant progress in this area, estimating the rate of celiac diagnosis to have increased dramatically in the U.K. over the last two decades, with nearly one in four sufferers now being diagnosed.[11] "People in this field were clapping themselves on the back when that study came out," says Sanders. "I think I clapped myself on the back too. But when I sat down and thought about it, I thought, that still means 75 percent of cases are undiagnosed. And that's absurd, isn't it?"

GRAINS OF TRUTH

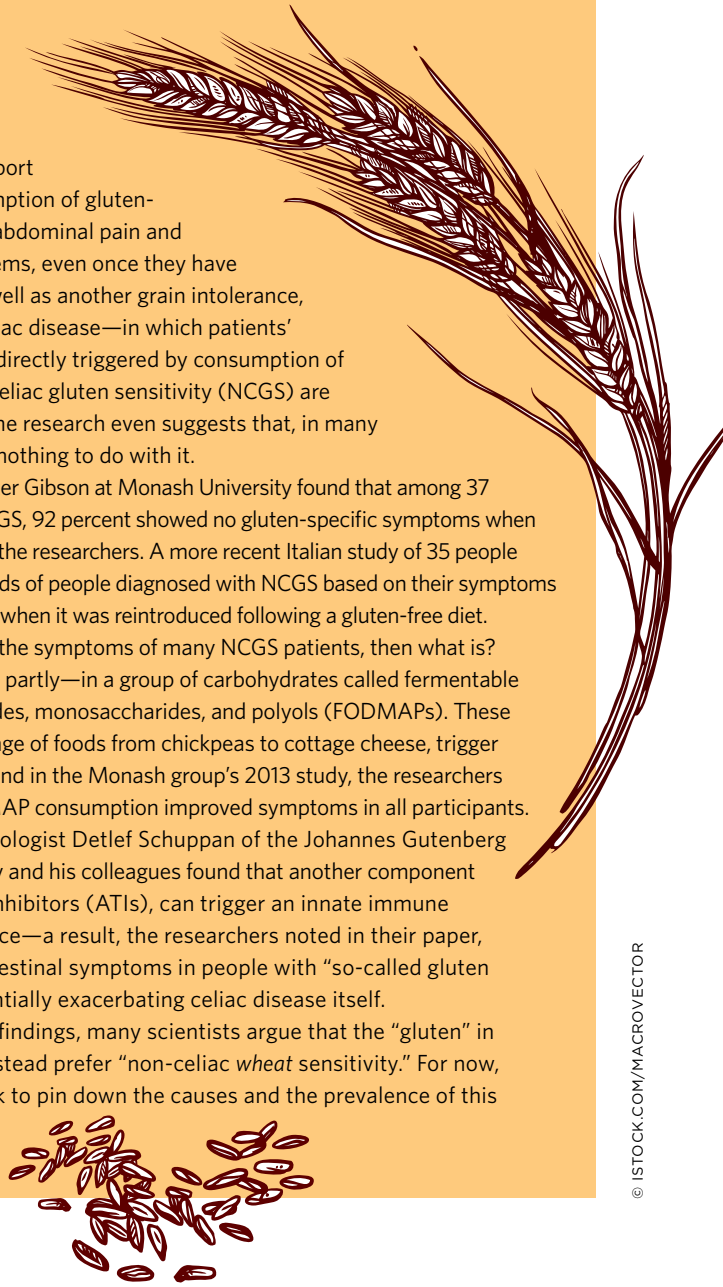
It's not just celiac patients who have a difficult relationship with gluten. Thousands of Americans report symptoms following consumption of gluten-containing foods, including abdominal pain and other gastrointestinal problems, even once they have ruled out celiac disease as well as another grain intolerance, wheat allergy. But unlike celiac disease—in which patients' symptoms are known to be directly triggered by consumption of gluten—the causes of non-celiac gluten sensitivity (NCGS) are far less understood, and some research even suggests that, in many patients, gluten might have nothing to do with it.

In 2013, a team led by Peter Gibson at Monash University found that among 37 people with self-reported NCGS, 92 percent showed no gluten-specific symptoms when following a diet controlled by the researchers. A more recent Italian study of 35 people found similar results: two-thirds of people diagnosed with NCGS based on their symptoms showed no reaction to gluten when it was reintroduced following a gluten-free diet.

But if gluten isn't behind the symptoms of many NCGS patients, then what is? The answer may lie—at least partly—in a group of carbohydrates called fermentable oligosaccharides, disaccharides, monosaccharides, and polyols (FODMAPs). These carbohydrates, found in a range of foods from chickpeas to cottage cheese, trigger gastrointestinal symptoms, and in the Monash group's 2013 study, the researchers showed that reducing FODMAP consumption improved symptoms in all participants.

Meanwhile, gastroenterologist Detlef Schuppan of the Johannes Gutenberg University Mainz in Germany and his colleagues found that another component of wheat, amylase trypsin inhibitors (ATIs), can trigger an innate immune response in humans and mice—a result, the researchers noted in their paper, that could explain gastrointestinal symptoms in people with "so-called gluten sensitivity," as well as potentially exacerbating celiac disease itself.

From these and related findings, many scientists argue that the "gluten" in NCGS is misleading, and instead prefer "non-celiac *wheat* sensitivity." For now, though, as researchers work to pin down the causes and the prevalence of this condition, the term sticks.



THERE'S A LOT OF NIHILISM TOWARDS THIS CONDITION, AND I THINK IT'S GOT A LOT TO DO WITH THE TREATMENT BEING A GLUTEN-FREE DIET. EVEN THE MEDICAL FRATERNITY CAN HAVE QUITE DEROGATORY VIEWS OF DIET-BASED TREATMENTS.

—David Sanders, University of Sheffield

Part of the delay in improvements has to do with medical practitioners "playing catch-up" with an expanding range of symptoms recognized as warning signs, Sanders adds. While traditionally linked to gastrointestinal symptoms such as diarrhea in children, celiac disease is increasingly being identified in older patients, many of whom don't display telltale gastrointestinal symptoms but have conditions previously deemed unrelated, such as anemia and osteoporosis. "The types of presentations have changed," agrees Murray, who published accounts of these nonclassic symptoms in US patients in the early 2000s. "It's turned on its head the preconceived notion of what the disease should be."

Sanders also points to reticence among physicians about diagnosing the condition—a situation that, somewhat paradoxically, hasn't been helped by the increasing popularity of gluten-free diets and publicity surrounding the "spectrum" of gluten-related disorders. (See "Grains of Truth" on opposite page.) "There's a lot of nihilism towards this condition, and I think it's got a lot to do with the [treatment being a] gluten-free diet," Sanders says. "If this was a tablet, nobody would argue, and physicians would say, 'Oh, you have to take tablet X.' But the word 'diet' has all sorts of connotations. . . . Even the medical fraternity can have quite derogatory views [of diet-based treatments]."

There's related concern that public attention to gluten could undermine how celiac disease is detected, as people self-prescribe a gluten-free diet even without being diagnosed by a doctor. "Now, the latest trend is people avoiding gluten without having a diagnosis at all," explains Murray. "I think that when it comes to counting and measuring and knowing what's happening in the world with celiac disease, it's going to be very hard to see that with this gluten-free train." Last year, for example, researchers at Rutgers University highlighted an apparent stabilization in celiac diagnoses since 2009, but also noted a soaring number of people following gluten-free diets in the U.S. without having ruled out celiac disease, making it impossible to determine the true fluctuation in celiac prevalence.[12]

To get a stronger grip on the global prevalence of celiac disease and better help those who are diagnosed, researchers are keen to discourage people from self-treating for gluten-related disorders. For all ailments thought to be related to gluten consumption, "there needs to be a greater awareness of science as a discipline to examine these questions," says Murray. "It doesn't matter how high you pile anecdotes, that does not science make." ■

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WHITE MULCH: Aerial view of farm land using plastic soil cover. Hubei province, central China.


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Planet Plastic

Contamination of marine and terrestrial ecosystems by microplastics is putting individual organisms at risk.

BY EE LING NG

White stripes, each about a meter wide, painted the land for as far as the eye could see. I was visiting the dry areas of the autonomous Ningxia region in northwest China with colleagues from the Chinese Academy of Agricultural Sciences and the nonprofit Centre for Agriculture and Biosciences International. Those strips of plastic sheeting, I was told, allow farmers to grow cash crops and grains despite the desert-like conditions. The sheets, usually composed of polyethylene, help conserve water, suppress weeds, and boost soil temperatures, effectively increasing crop yields by 20 to 60 percent.

The use of plastic "mulch" to grow crops, known as the White Revolution, began in China in the late 1970s, and now covers 20 million hectares of the country's agricultural land, an area equivalent to half the size of California. Other countries in the Middle East, Europe, and North America also use plastic mulch.

Used sheets are expensive to collect and discard or recycle, leading some farmers to leave them on the field or illegally dump or burn them; often the sheets are not fully removed, as the thin plastic readily tears into small fragments that remain on the farmland, forming what is known in China as "white pollution." As new plastic mulch is applied year after year, soils can become enriched with plastic residues, which change the physical and chemical properties of the habitat that so many plants, animals, and microorganisms call home.

The effects of plastic pollution in terrestrial environments remain largely unknown. To date, the majority of research has focused on aquatic systems, as 10 million to 20 million tons of plastic litter find their way to the oceans each year.

Starting in the early 1960s, researchers began documenting dead seabirds with stomachs full of plastic. Then, in 1997, racing boat captain and oceanographer Charles Moore captured the public's attention with his accounts of floating plastic debris in an area now known as the Great Pacific Garbage Patch, ranging from the West Coast of North America to Japan. In addition to large plastic trash, Moore described tiny colorful fragments, what are today known as microplastics, suggesting that a subtler pollutant was accumulating in the environment.

In addition to resulting from the physical fragmentation of larger plastic products, microplastics—loosely defined as particles in the size range of 100 nanometers to 5 millimeters—are introduced into the environment as a result of their use in a wide range of applications, from personal-care products to industrial abrasives employed in paint removal or cleaning. Nanoplastic particles of less than 100 nanometers have also found a wide range of applications in adhesives, paints, electronics, and more.

Marine organisms across all trophic levels have turned up with microplastics in their guts, and evidence is emerging from experimental studies that the plastic particles and the cocktail of chemicals they carry can wreak havoc on physiology, reproduction, development, and behavior in a range of species.

A steady diet of plastic

Estimates of nano- and microplastic loading in terrestrial environments remain sparse, in part hindered by the heterogeneous nature of soil. But substantial levels of plastic pollution can be expected. Farmlands may be at particularly high risk.

The use of plastic "mulch" to grow crops, known as the White Revolution, began in China in the late 1970s, and now covers 20 million hectares of the country's agricultural land.

Even in areas that don't employ plastic mulching, farmers may apply microplastics-ridden sewage sludge as fertilizer, and treated wastewater is an important source of water for irrigating farmland.

Last year, estimates by a group of Scandinavian and Czech researchers suggested that we could be inadvertently adding some 44,000 to 300,000 metric tons of microplastics annually to farmlands in North America, and another 63,000 to 430,000 tons in Europe. The quantities from each region alone, the researchers said, would exceed the estimated 93,000 to 236,000 tons of microplastics in the surface water of all Earth's oceans.[1] And new studies of terrestrial environments are showing that, like marine animals, soil organisms ingest these microplastics.

While humanity moves slowly on addressing the plastics conundrum, small aquatic organisms are already making themselves at home on microplastic debris. In 2014, Julia Reisser of the University of Western Australia and her colleagues found that a diversity of algae, marine worms, barnacles, insect eggs, and microbes inhabit microplastics floating in the oceans surrounding Australia.[2] Animals higher up in the food web that eat these organisms ingest the plastics along with the prey, setting the potential for harm to reverberate through the ecosystem.

Last November, Matthew Savoca and his colleagues at University of California, Davis, provided evidence that the plastics may smell good to certain consumers. The researchers placed plastic beads in mesh bags tied to an oceanographic monitoring system, then recovered the beads after three weeks in the ocean. The team found that the beads emitted dimethyl sulfide—a compound produced by phytoplankton in response to grazing by zooplankton, which in turn attracts predators of the zooplankton. Using field data collected from 13,350 seabirds of 25 Procellariiform species, including albatrosses and petrels, the researchers found that bird species known to be attracted to dimethyl sulfide ingested the greatest amounts of plastic.[3]

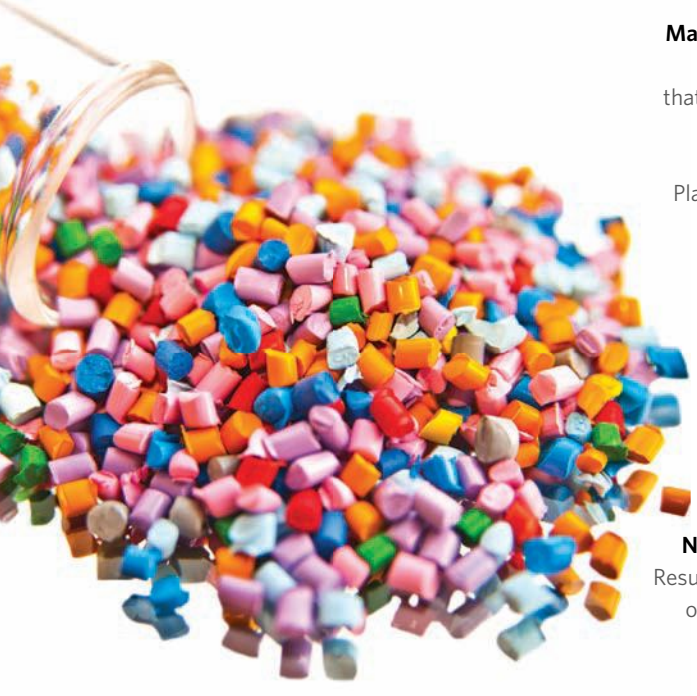
The growing levels of microplastics in the environment—both aquatic and terrestrial—should thus be cause for concern. As plastic particles get smaller, a wider range of organisms is at risk of uptake or ingestion, and at the nanoscale, the particles can enter cells and move beyond the gastrointestinal system.

Scientists' understanding of the scale of the problem, in terms of both how much plastic pollution exists in the environment and the nature of the consequences for organisms and ecosystems, remains woefully inadequate.

The environmental toll

When examining the effects of plastic on the environment, researchers must assess not just the plastic polymer itself, which is

TYPES OF PLASTIC POLLUTION



Macroplastics: Greater than 5 cm
Plastic trash and large debris that results from its fragmentation

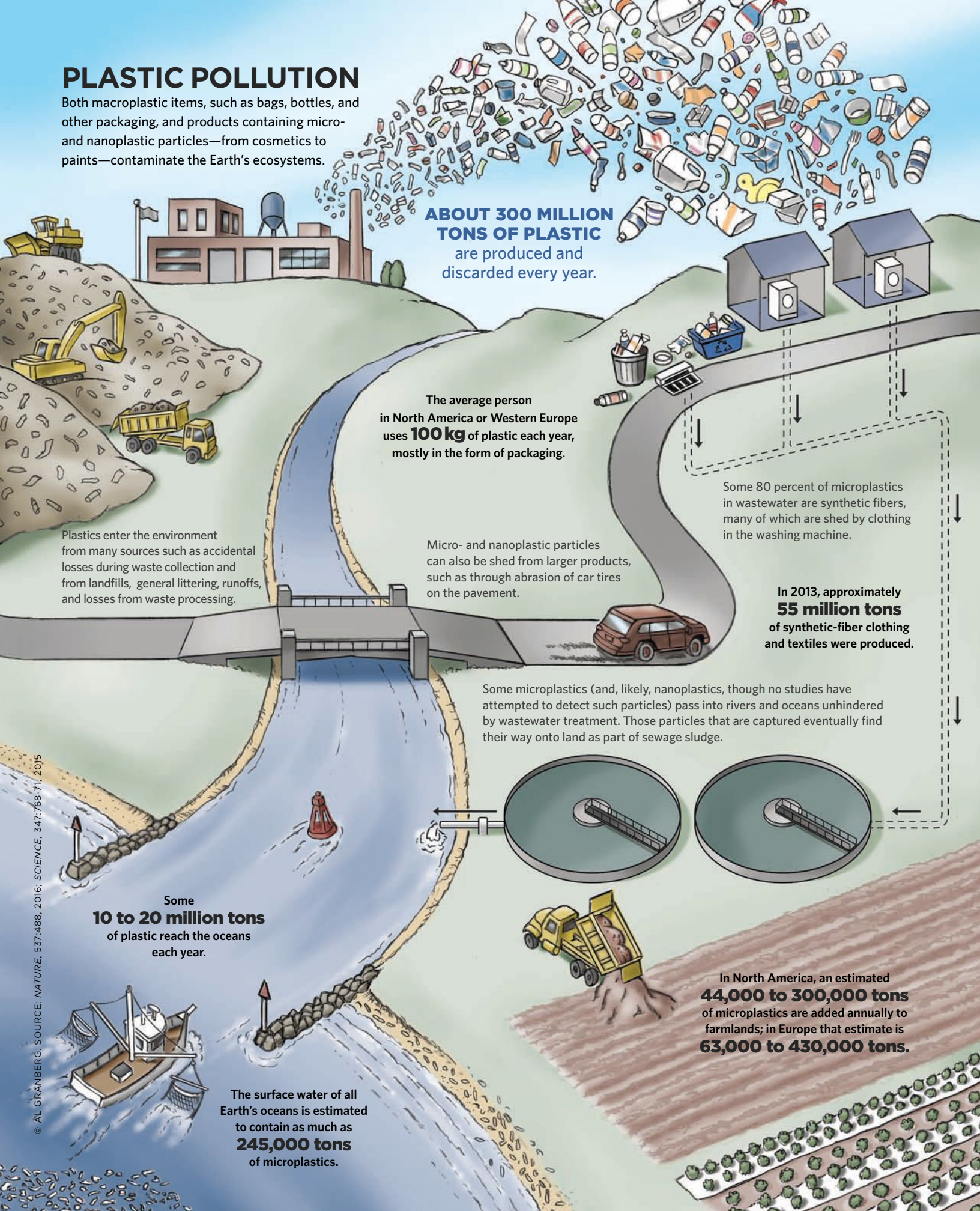
Mesoplastics: 5 mm–5 cm
Plastic trash and large debris that results from its fragmentation

Microplastics: 100 nm–5 mm
Results from general wear-and-tear of plastic materials, including washing synthetic fabrics and abrasion of car tires; also used in diverse products

Nanoplastics: Less than 100 nm
Results from general wear-and-tear of plastic materials; increasingly used in diverse products

PLASTIC POLLUTION

Both macroplastic items, such as bags, bottles, and other packaging, and products containing micro- and nanoplastic particles—from cosmetics to paints—contaminate the Earth's ecosystems.



ABOUT 300 MILLION TONS OF PLASTIC are produced and discarded every year.

The average person in North America or Western Europe uses 100 kg of plastic each year, mostly in the form of packaging.

Some 80 percent of microplastics in wastewater are synthetic fibers, many of which are shed by clothing in the washing machine.

Plastics enter the environment from many sources such as accidental losses during waste collection and from landfills, general littering, runoffs, and losses from waste processing.

Micro- and nanoplastic particles can also be shed from larger products, such as through abrasion of car tires on the pavement.

In 2013, approximately 55 million tons of synthetic-fiber clothing and textiles were produced.

Some microplastics (and, likely, nanoplastics, though no studies have attempted to detect such particles) pass into rivers and oceans unhindered by wastewater treatment. Those particles that are captured eventually find their way onto land as part of sewage sludge.

Some 10 to 20 million tons of plastic reach the oceans each year.

In North America, an estimated 44,000 to 300,000 tons of microplastics are added annually to farmlands; in Europe that estimate is 63,000 to 430,000 tons.

The surface water of all Earth's oceans is estimated to contain as much as 245,000 tons of microplastics.

generally considered inert, but the various chemicals (called additives) mixed in during the manufacturing process. A cocktail of plasticizers, such as phthalates, as well as flame retardants, stabilizers, pigments, and antimicrobials can improve the product's properties, such as transparency, flexibility, and durability.

Plastics also act as magnets for hydrophobic, or water-repelling, organic and inorganic pollutants, courtesy of their chemical and physical properties. Persistent organic pollutants, such as dioxins, polychlorinated biphenyls (PCB), dichlorodiphenyltrichloroethane (DDT), and polycyclic aromatic hydrocarbons (PAH), have been detected hitchhiking on marine plastics.

Researchers have begun to document the toll of plastic additives and organic pollutants carried by microplastics on aquatic animals. Once plastics have been consumed, some of the chemicals are released from the plastic and transferred to the animal. If the chemical is fat soluble, it may accumulate in an organism's tissues.

In one experiment, an international research team fed shearwater chicks in Tokyo Bay PCB-contaminated polyethylene resin pellets, and later measured increasing levels of PCB in the birds' preen gland oil.[4] And laboratory experiments using rodent models have shown that exposure to plastic additives can disrupt the endocrine system, cause birth defects, reduce sperm production, trigger insulin resistance, and impair learning and memory.[5]

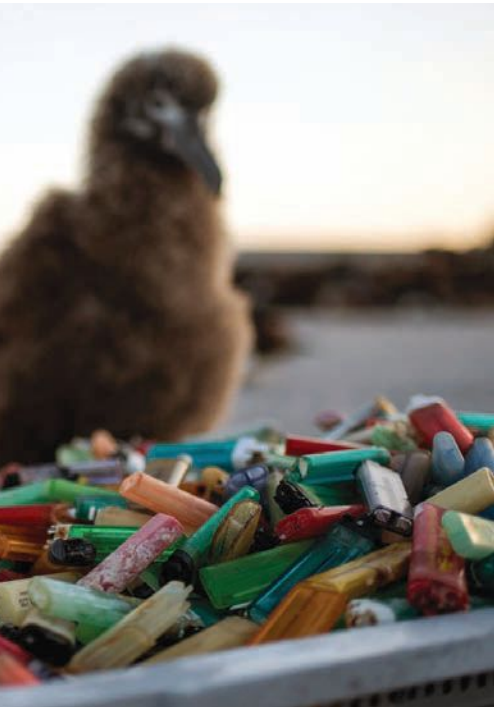
Research on the terrestrial environment is only starting, decades behind that on the marine environment. On land, nearly a half century of plastic mulching has provided initial insights into the terrestrial ecosystem's plastic burden. Last year, for example, a team of researchers in China found that soil microbial biomass and the microbes' overall metabolic activity decreased with increasing plastic mulch residues in the soil. The microbes' functional diversity, as measured by their use of different carbon sources, was also reduced.[6] Also last year, Esperanza Huerta Lwanga, a visiting researcher at Wageningen University and Research Center in the Netherlands, and colleagues found that earthworms fed microplastic-tainted plant litter grew more slowly and died earlier, although reproduction was unaffected.[7] Another study, published this January, pointed to increased gut inflammation in microplastic-exposed worms.[8]

Huerta Lwanga and her colleagues have also shown that earthworms play a role in transporting microplastics from the surface of the soil deeper into the ground. In experiments using thin boxes called formicaria that allow observation of the tunnels made by earthworms, the researchers found microplastics along the tunnels. A study published in March reported that springtails, a highly abundant group of microarthropods, can also transport microplastics in the soil.[9] This movement of microplastics presumably makes the pollutants accessible to other soil organisms, though the possibility for bioaccumulation along the soil food web has yet to be determined.

On land, the lifetime of plastics is estimated to be in the range of centuries. Because we currently have no way to get rid of them, microplastics will continue to persist and accumulate in soil and water.

If plastics are making their way through terrestrial ecosystems, the risks to plants, which form the base of many terrestrial food webs, must be assessed. Microplastics are not expected to be a problem for plants, as their large molecular weight would prevent them from passing through plant cell walls. Nanoplastics, however, can and do get inside plant cells. A study using tobacco plant cells showed that nanopolystyrene beads of 20 to 40 nm were taken up, while beads of 100 nm were not.[10] More research is needed to determine how plants are affected, however, and whether there are any knock-on effects in the ecosystem.

TOP LEFT AND BOTTOM LEFT: NOAA
TOP RIGHT: VBERGER/WIKIMEDIA COMMONS



AWASH IN PLASTICS: Discarded plastics that have made their way into the world's oceans include cigarette lighters (top left), empty water bottles (top right), and tangles of fishing line (bottom left). The guts of sea birds contain both undegraded items and microplastic particles (bottom right).

BOTTOM RIGHT: CHRIS JORDAN/U.S. FISH AND WILDLIFE SERVICE HEADQUARTERS

Plastics, plastics everywhere

Back in China, shipping containers full of plastic arrive at thousands of processing centers, ranging from small, family-run businesses to large operations. Almost half of recycled plastic waste in the U.S., Europe, and Australia is exported, and China is its biggest importer, using old plastic to make new products.

China is the largest producer of plastic products. In the award-winning documentary *Plastic China,* released in January of this year, director Wang Jiuliang captured images portraying the human and environmental costs of the plastic recycling trade: children playing amidst hills of trash, standing under a shower of fluffy plastic fibers, and using discarded surgical gloves as balloons.

While the questions of environmental impacts and human health risk remain open (see "The Human Consequence" on page 47), the world continues to rely heavily on plastics that pollute the environment. Some 8 percent of global oil production is used in plastic manufacturing, both as the raw material and for energy to power the process. In Australia alone, 1.4 megatons of plastics were used during the

PLASTICS OVERLOAD: The sheer quantity of plastics and the material's durability poses enormous challenges to waste recycling.

2011 to 2012 financial year. That is equivalent to the mass of some 1 million SUVs consumed by a country with a population of fewer than 23 million people.

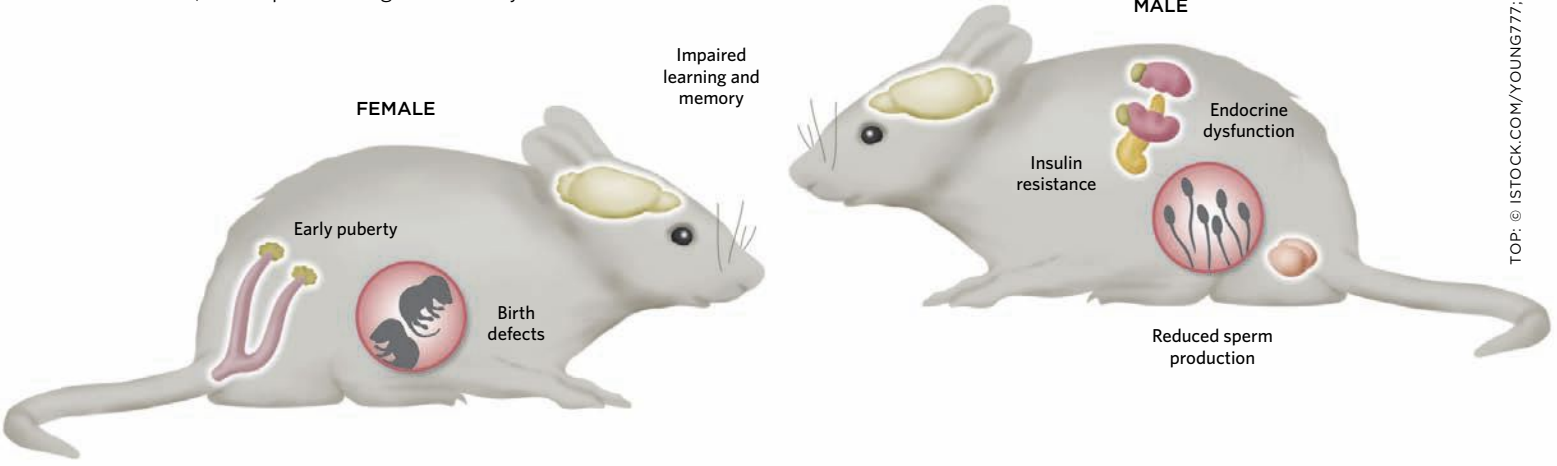
Ironically, durability, the characteristic that has made plastic so useful to humans, is a double-edged sword. A stark reminder of how persistent plastics are and how far they can travel from their point of origin came in 2005, when an albatross was found with plastic debris in its guts from a World War II plane that had crashed in the sea some 9,000 kilometers away six decades earlier. On land, the lifetime of plastics is estimated to be in the range of centuries. Because we currently have no way to get rid of them, microplastics will continue to persist and accumulate in soil and water.

So, what is the best thing to do based on current knowledge? We know that microplastics do not degrade easily in the environment. We know that nanoplastics can enter cells. We have observed that nano- and microplastics are ingested or taken up in a range of marine and terrestrial organisms and that some pollutants carried by microplastics are transferred from food to these consumers, with adverse effects.

With such high stakes, we must engage in existing solutions rather than wait for definitive answers. As stated in the 1998 Wingspread Statement on the precautionary principle, "When the health of humans and the environment is at stake, it may not be necessary to wait for scientific certainty to take protective action." Our priority should be to eliminate the unneces-

PLASTICS' EFFECTS

Research into the consequences of plastic pollution for organisms living in contaminated environments remains sparse. The most extensive work done so far has focused on plastic additives, such as bisphenol A, polybrominated diphenyl ethers, and phthalates. Laboratory experiments using rodent models have demonstrated that these compounds can disrupt the endocrine system, cause birth defects, reduce sperm production, trigger insulin resistance, and impair learning and memory.



TOP: © ISTOCK.COM/YOUNG777; BOTTOM: © AL GRANBERG

sary use of plastics, to improve and insist on biodegradable alternatives, and, above all, to fully understand what it means to live in a plasticized world. ■

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THE HUMAN CONSEQUENCE

As plastic pollution invades Earth's numerous ecosystems, it should come as no surprise that small particles and plastic additives have found their way into the human food supply.

In 2014, Lisbeth Van Cauwenberghe and Colin Janssen of Ghent University in Belgium tested for microplastics in two filter-feeding bivalve species grown for human consumption—blue mussels (*Mytilus edulis*) and Pacific oysters (*Crassostrea gigas*). They found levels of microplastics in the animals' tissues that would translate to human dietary exposure of between 1,800 and 11,000 microplastic particles per year for Europe's minor and top mollusk consumers respectively.[11]

A 2015 survey of 44 samples of 10 vegetables (capsicum, cucumber, Chinese cabbage, radish, green cabbage, lettuce, chrysanthemum, celery, spinach, and mustard) grown with plastic mulch in suburban greenhouses around Nanjing, China, documented the presence of a group of phthalates in all 44 samples.[12] Several had concentrations that exceeded allowable limits for human consumption or for soils set by both European and US governments. Microplastics have even been found in salt, unsurprising given that our seawater is being enriched with them.[13]

To date, no guidelines or regulations exist to limit the level of microplastics found in food products. And whether consuming these plastics will have effects on human health remains to be seen. Documenting such effects will require tracing each step along the pathways of exposure; food consumption is only one of those steps. It will also require research to reveal in greater detail how plastics, plastic additives, and other chemicals hitchhiking onto plastic during its residence in the environment act inside the body.

We do know that plastic additives such as phthalates are already widespread in human populations, based on monitoring in the U.S. and Europe. And human epidemiological studies have revealed links between plastic additives and metabolic, thyroid, reproductive, and respiratory problems.[14] But the pathways of exposure to these plastic additives are numerous, and ingestion of microplastics, based on current knowledge, is not expected to be the major contributor of these plastic additives found in people. Indeed, in a report on nano- and microplastics in food released in May 2016, the European Food Safety Agency (EFSA) Panel on Contaminants in the Food Chain stated that, while there is insufficient data for a complete human risk assessment, current evidence indicates microplastics in food are unlikely to be harmful.

The Literature

EDITOR'S CHOICE IN GENETICS & GENOMICS

Exome-Tailored Diets

THE PAPER

M.D.W. Piper et al., "Matching dietary amino acid balance to the in silico–translated exome optimizes growth and reproduction without cost to lifespan," *Cell Metabolism*, 25:610–21, 2017.

Animal studies indicate that while calorie restriction prolongs life, a protein-rich diet can shorten it, even if overall calories are low. Earlier in life, however, higher protein consumption is required for reproductive fitness. According to evolutionary theory, "reproduction and lifespan are locked into a trade-off with each other: you do one well, and do the other poorly," says Matthew Piper of Monash University in Melbourne, Australia.

Piper and colleagues had previously found that tinkering with the proportions of certain amino acids in the diet of fruit flies could somewhat redress this fecundity-longevity imbalance. And this led Piper to ask if it would be possible to construct a diet that enhances both fecundity and longevity.

In a moment of inspiration, Piper considered that the recipe for balanced amino acid consumption might be written in the genes—specifically, in the protein-coding exome. To test his theory, Piper designed a diet for fruit flies in which the amino acid component precisely reflected the proportional abundance of each amino acid specified by the insect's exome. He then compared the behavior and physiology of flies on this exome-matched diet to those eating diets that were equivalent in calories and nutrient proportions but not in amino acid ratios.

When given a choice, the flies preferred the exome-matched diet to the unmatched diets. Interestingly, when allowed to eat ad libitum, flies on the exome-matched diet ate less than those on the unmatched diets, suggesting the former provided greater satiety and nutritional efficiency. Flies on the exome-matched diet also exhibited increased growth rate, adult body mass, and fecundity (egg laying) compared to those on the unmatched diets, while longevity was unaltered. Similar effects on growth and satiety were seen in mice fed an exome-matched diet.

This is "innovative and important" work, says Andrzej Bartke of Southern Illinois University. "[It] show[s] that genetic information predicts the utilization of amino acids by an organism, and that you can therefore design a diet based on the organism's genes."

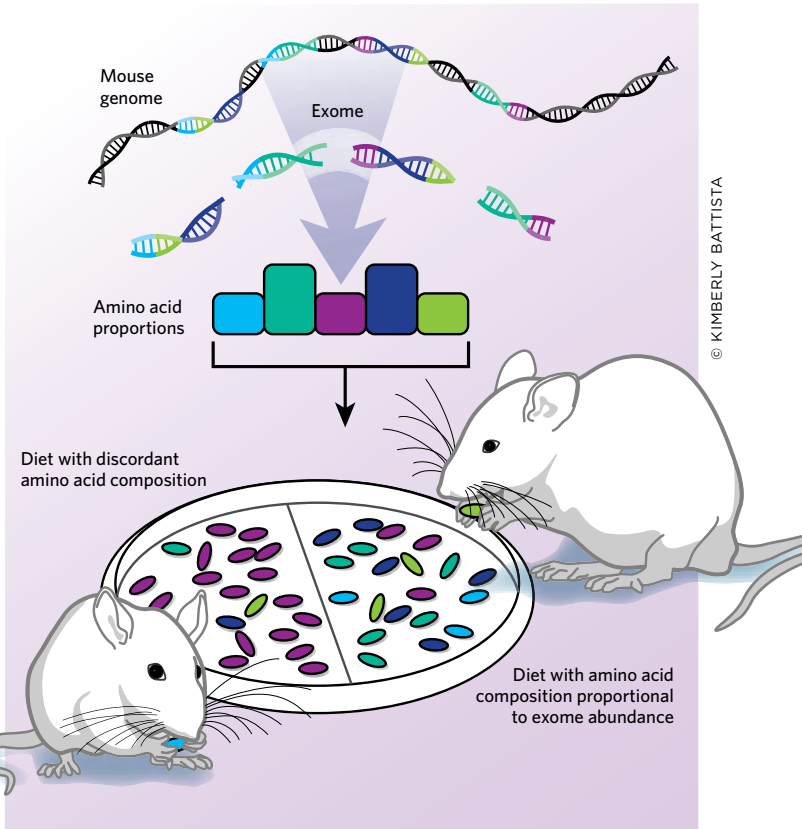
Furthermore, Bartke adds, "most of the fundamental mechanisms linking nutrients to growth, reproduction, and aging are remarkably similar in widely different species. It is therefore very reasonable to expect that these findings are relevant to human nutrition."

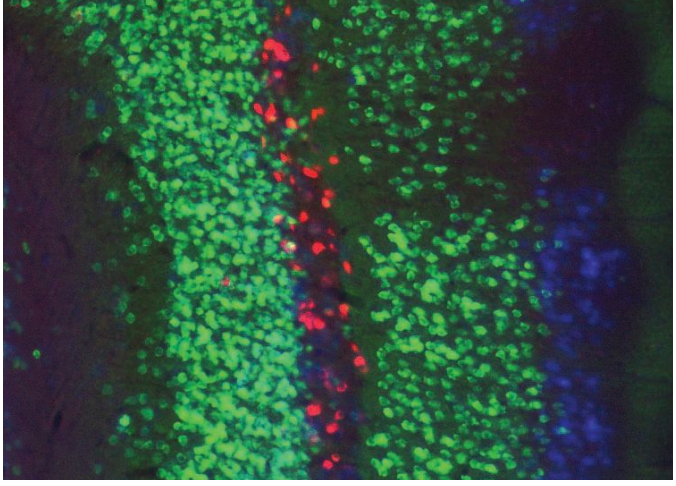
That said, Piper hastens to point out that the animals in the study were kept in a temperature- and humidity-controlled infection-free environment. "What we're looking into now is whether [the results are] relevant outside of lab conditions," he says.

Even if such exome-tailored diets are not applicable, or desirable, for humans, says James Mitchell of Harvard T.H. Chan School of Public Health, "there's a huge interest for industrial animal husbandry" where the goal is to maximize growth and, in the case of chickens, egg-laying, "but also to save money on the feed."

—Ruth Williams

DNA-TO-DISH RECIPE: To generate diets with species-specific proportions of amino acids, first sequence all the protein-coding regions (the exome) from an organism's genome and determine the precise proportion of each of the 20 encoded amino acids (five of the 20 are shown as colored codons in DNA, with their respective abundances displayed in the bar chart). Next, mix amino acids at this exact ratio with other nutrients (carbohydrates, lipids, and so on) and serve. Animals on this exome-matched diet will grow larger than those on equivalent diets that have discordant amino acid compositions.





RECORDERS: Entorhinal cortex cells (red) that project to the prefrontal cortex are important for memory in mice.

NEUROSCIENCE

Memory Maturation

THE PAPER

T. Kitamura et al., "Engrams and circuits crucial for systems consolidation of a memory," *Science*, 356:73-78, 2017.

MEMORY THEORIES

The theory goes that as memories form, they set up temporary shop in the hippocampus, a subcortical region buried deep in the brain, but over time find permanent storage in the cortex. The details of this process are sketchy, so Takashi Kitamura, a researcher in Susumu Tonegawa's MIT lab, and colleagues wanted to pinpoint the time memories spend in each of these regions.

TOTAL RECALL

As mice were subjected to a fearful experience, the team labeled so-called memory engram cells—neurons that are stimulated during the initial exposure and whose later activity drives recollection of the original stimulus (in this case, indicated by a freezing response). Using optogenetics, Kitamura turned off these cells in the prefrontal cortex (PFC) when the memory first formed as mice were exposed to a foot shock. Short-term memory was unaffected, but a couple of weeks later, the animals could not recall the event, indicating that PFC engrams formed contemporaneously with those in the hippocampus, not later, as some had suspected, and that this early memory trace in the cortex was critical for long-term retrieval.

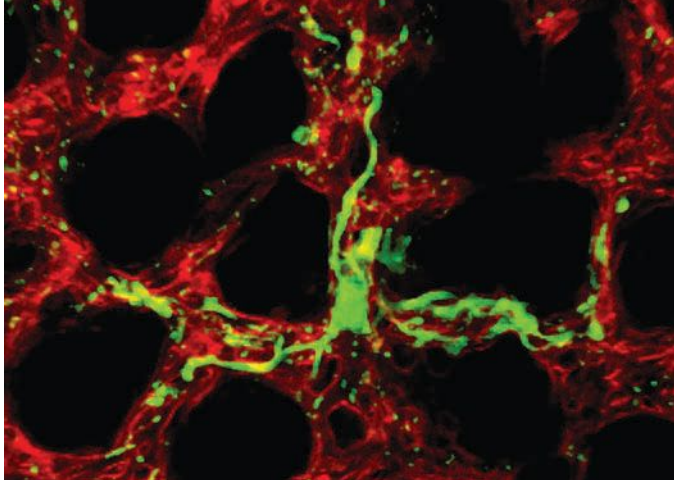
GOING DARK

Over time, as untreated mice recalled the fearful event, engrams in the hippocampus became silent as PFC engrams became more active. "It's a see-saw situation," says Kitamura, "this maturation of prefrontal engrams and dematuration of hippocampal engrams."

CIRCUIT DYNAMICS

Stephen Maren, who researches memory at Texas A&M University and was not part of the study, says the results reveal that the network circuitry involved in memory consolidation (of which Kitamura's team dissected just one component) is much more dynamic than previously appreciated. "It's the most sophisticated circuit-level analysis we have to date of these processes." —Kerry Grens

TAKASHI KITAMURA, TONEGAWA LAB; COURTESY OF MARK LOONEY, UCSF



PLATELET RELEASE: In the small blood vessels (red) of a mouse lung, megakaryocytes (green) pinch off their cytoplasm to form platelets.

PHYSIOLOGY

Pulmonary Platelets

THE PAPER

E. Lefrançais et al., "The lung is a site of platelet biogenesis and a reservoir for haematopoietic progenitors," *Nature*, 544:105-09, 2017.

LUNG INTRIGUE

Platelets form when megakaryocytes release bits of their specialized cytoplasm, a process long thought to occur primarily in the bone marrow. Mark Looney, a pulmonologist at the University of California, San Francisco, says human studies had reported that blood leaving the lungs contained fewer megakaryocytes and more platelets than blood entering it—suggesting the lungs were producing platelets. "But it's never been visualized, it's never been caught in the act."

THE MAGNITUDE

In a recent study, Looney and colleagues observed fluorescently labeled megakaryocytes in mice releasing platelets within the lung microvasculature—catching "the act" on video. The researchers estimated that a mouse's lungs produce about 10 million platelets per hour—half of the animal's total platelet production.

THE SOURCE

When the researchers transplanted a normal lung into a mouse with labeled megakaryocytes, glowing megakaryocytes and platelets soon appeared in the transplanted lung. Moving a lung with fluorescently labeled cells into a normal mouse, however, did not yield glowing platelets. This result indicates that the productive megakaryocytes originated outside the lung—in the bone marrow, the researchers believe.

HUMAN CONSEQUENCES

It's unclear if human lungs are significant platelet sources, says UCSF hematologist Jack Levin. If so, "one would have to anticipate that at least some pulmonary disorders would be associated with a reduced platelet count," or thrombocytopenia. Yet Levin knows of no such examples. Looney counters that the lung damage in common pulmonary conditions is too small to cause thrombocytopenia, but that the condition is associated with surgeries in which a machine takes over for the heart and lungs temporarily and lung circulation is bypassed. —Ashley P. Taylor

Micronutrients, Macro Impact

At the interface of food, nutrition, and agriculture, Lindsay Allen's research has been informing nutrition guidelines and policies around the world for decades.

BY ANNA AZVOLINSKY

Lindsay Allen landed in California from England in 1969, following her then partner, a graduate student at the University of California, Davis. She had received a bachelor's degree in food science and nutrition two years earlier and then worked in a human nutrition lab at the University of Cambridge. Allen began working as a technician in the UC Davis food science lab of Frances Zeman, who suggested that she should pursue a PhD. "I never considered getting a PhD or having an academic career, because few people in the U.K. went to graduate school, which was incredibly specialized there," says Allen, who now serves as the director of the US Department of Agriculture Agricultural Research Service's (ARS) Western Human Nutrition Research Center in California.

Allen enjoyed the coursework and found that her academic background—a science-focused curriculum of chemistry, biology, and nutrition courses in high school and during university in England—had prepared her well for graduate school.

> "We are trying to figure out how and why different people respond differently to food interventions."

For her thesis, Allen worked with a rat model to understand how protein deficiency during pregnancy affected female animals and their offspring. "At the time, there was a lot of interest in protein deficiency as a major cause of human malnutrition," she says. Compared to the progeny of control rats that were not deficient in protein intake during gestation, the progeny of deficient animals had permanent changes in their body composition and in kidney development, Allen found. She is quick to point out that our knowledge of malnutrition has evolved significantly since her early rat work: "I'm not sure how relevant that model was for humans because people are never just deficient in protein." Still, the work was related to human research conducted later by British epidemiologist David Barker that culminated in his eponymous 1990 hypothesis proposing that pre- and postnatal undernutrition, low birth weight, or premature birth can contribute to the development of chronic disorders such as diabetes and coronary heart disease later in life.

Even before her graduate school days in Zeman's lab, Allen had been asking questions about what constitutes malnutrition and undernutrition in people, and has continued to probe its causes and consequences for more than 30 years. Allen's human nutrition work in the U.S. and internationally in countries such as Mexico, Guatemala, and Kenya has resulted in nutrition policy changes she herself helped to write.

Here, Allen talks about her working-class roots, her seminal human studies showing that deficiencies in vitamin B12 and other animal-sourced micronutrients are at the heart of many poor-quality diets around the world, and why research on human milk has been slow to develop.

ALLEN ACTS

Strong work-ethic roots. Allen was born and raised in Clevedon, Somerset, a town in southwestern England on the Bristol Channel. "I come from a lower-middle-class background. My great-grandfather and grandfather were bakers who had their own business. They worked night and day. I grew up exposed to rural, working-class England, for which I am very grateful. Those values and experiences have carried over to the rest of my life," she says.

Seminal experiences. In 1964, she entered the University of Nottingham, about 150 miles northeast of her hometown. There, she chose to focus on agriculture, food science, and nutrition, which exposed her to many different areas of science. While at university she did everything from horticulture and working in potato fields to analyzing the nutritional composition of packaged foods on the shelves of stores in the U.K. "There were huge nutrient losses from the production processes. What the label said on the package was not what was in the box."

Inspiring mentor. After graduating in 1967, Allen worked in dietitian Elsie Widdowson's lab at the University of Cambridge. Widdowson, among the most famous female scientists in the U.K. at the time, had designed the diets for British troops during World War II and studied how wartime rationing affected health. "She did incredibly pioneering research and was just an amazing woman. She showed me what was possible and reinforced my interests in studying human nutrition," says Allen. In Widdowson's lab, Allen made her first foray into human nutrition research: she helped conduct studies on the composition of human breast milk from women in the U.K. to understand how to improve infant formulas.

Nutritious beginnings. After receiving a PhD from UC Davis in 1973, Allen did a postdoc in the nutrition department at the University of California, Berkeley. She found that higher dietary protein consumption caused increased calcium loss from the body. "I worked in a human research facility called 'the penthouse.' It was

PHOTO BY MAYUMI ACOSTA PHOTOGRAPHY

LINDSAY ALLEN

Director, US Department of Agriculture (USDA)
Agricultural Research Service (ARS) Western Human Nutrition Research Center (WHNRC)
Research Professor, Department of Nutrition
University of California, Davis

Greatest Hits

- Showed that a diet deficient in animal-sourced foods, rather than low calorie intake, is a cause of stunted growth and poor development in children
- Among the first to uncover that deficiencies in vitamins and/or minerals are a major reason why consumption of poor-quality diets negatively affects pregnancy, lactation, and child growth and development
- Among the first to uncover the high prevalence of vitamin B12 deficiency around the world and to identify the causes and consequences of the deficiency, leading to vitamin B12 food-fortification programs in developing countries
- Established global guidelines for adding micronutrients to flour and other foods
- Developed efficient methods for analyzing nutrient content in human breast milk that revealed low concentrations of milk micronutrients in mothers consuming low-quality diets

three rooms that could house six people. We had paid volunteers live there for four months at a time, and we'd feed them formula diets with exactly known nutritional composition. Since this was Berkeley in the 1970s, we would get really interesting people who were willing to live like that for four months!" Allen says.

ALLEN ADVANCES

Getting her feet wet. In 1974, Allen became an assistant professor at the University of Connecticut, where she began to do the human population studies she had always wanted to conduct. "The funding for human research studies was really hard to come by in those days," she says. She and her colleagues studied the effects of diet, stress, and cigarette smoking on pregnancy outcomes in that state, finding that maternal low weight gain and smoking independently resulted in negative effects on babies, including lower birth weight and impaired postnatal motor performance and reflexes. Then, in 1981, Allen began what she calls her "real career," working with colleagues at the Salvador Zubirán National Institute of Nutrition in Mexico City on the Mexico Collaborative Research Project to study malnutrition in rural Mexico. "I had almost no field experience or any knowledge of Spanish at the time and was handed a multimillion-dollar project to manage," says Allen. The work, which took about eight years and included a yearlong sabbatical in Mexico and many international collaborators, was the start of Allen's research on the prevalence of specific micronutrient deficiencies, how populations were affected, and the design of interventions to prevent and treat those deficiencies.

Important hints. While in Mexico, Allen collaborated with Adolfo Chávez, who had initially found evidence of stunted growth and poor diet in rural parts of the country. "Our research together ended up being a really good base that led to our discovery that lack of micronutrients was the primary basis of malnutrition. The diets in most of Mexico at that time were very low in animal-source foods, and based primarily on maize, legumes, and pasta, but not rich in many micronutrients," says Allen. She and her colleagues followed Mexican women and their children aged 18 to 30 months and 7 to 8 years. The many measurements included detailed food intake, growth, and body weight, among other factors. Despite prior theories that attributed stunted growth to low overall calorie or protein intake, better growth during early childhood correlated not with a diet high in calories or proteins, but rather with a diet richer in animal-origin food, Allen found. "In the 1970s, the theory was that growth stunting happened because children didn't have enough food to eat. While this is true in some children, energy deficiency, we found, is not the main

problem. If you don't have enough energy you will gradually wither away and die, but what we were seeing was different," says Allen.

Missing micronutrients. "Then serendipitously, we started measuring the micronutrients in the blood of the subjects we were following and discovered really low levels of vitamins B2, B12, A, and E, and minerals such as iron and zinc. That was one of the first clues that the real problem with low dietary quality was lack of these micronutrients," says Allen. She and her colleagues were among the first to uncover that deficiencies in micronutrients were prevalent in rural Mexico. "For most people there, maize and beans were the staples, and there was not enough access to animal-sourced foods, which led to not enough B vitamins. Dietary quality was really strongly associated with almost every outcome we looked at, from infant growth to child cognitive function to school performance."

Implementing change. Allen was particularly interested in deficiencies of vitamin B12, which is only found in animal products. In follow-up studies in other countries, including Kenya, Allen and her team of collaborators showed that supplementation with milk or meat improved vitamin B12 levels in school-age children and that consumption of these foods improved cognitive function and growth in these children. Her research on micronutrient deficiencies affected policy changes implemented by the World Health Organization (WHO) and other international agency–led food-fortification projects. "About 90 countries now fortify wheat and rice flour with vitamins and minerals, and this is still an evolving process," says Allen, who was one of the authors of the 2006 WHO Guidelines on Food Fortification with Micronutrients. "There is now an interest from the Food Fortification Initiative to add vitamin B12 to certain foods, and I think our research is partly responsible for creating that interest," says Allen.

ALLEN ACCOMPLISHES

Brain effects. In 1993, Allen moved her laboratory from Connecticut to UC Davis, and in 2004 became the director of the USDA's ARS Western Human Nutrition Research Center. Following up on her earlier work on vitamin B12 deficiency work in women and children, Allen's team recently found, in a population study in Chile, that vitamin B12 deficiency in the elderly results in less efficient nerve conduction due to poor myelination of peripheral nerves, and that supplementation with the vitamin can lead to neurological improvements. "What I always wanted to do was to follow up supplementation with cutting-edge tracking methods to understand the changes that supplementation can have. This is exactly the kind of work we can do at the WHNRC. In the study in Chile, we looked at hundreds of metabolites before the vitamin B12 injections and then again four months later. That gave us clues that there were phospholipids in the blood that increased in response to vitamin B12 that we never would have known about, which make up a major percentage of the phospholipids in nerve tissue in the brain. This agrees with the fact that vitamin B12 deficiency can result in severe neurological and cognitive problems and loss of brain and nerve tissue that are related to lack of myelination," says Allen.

Pioneering methods. Allen's lab has also developed a novel way to analyze the B vitamin content in human milk from a single small sample. Breast milk analyses have lagged behind, according to Allen, because of the risk of misinterpreting the results and concluding that women should not breastfeed exclusively. "There is clear evidence that women should exclusively breastfeed for the first six months. That is the best advice. So any studies that show some breast milk may not be high quality risk creating confusion." The second reason is that because of the carbohydrates, fats, and proteins that form in the matrix of breast milk, it is more difficult to study than human serum using mass spectrometry. After assessing how well fortification and supplementation increases micronutrient content in breast milk, Allen is now working on a Bill & Melinda Gates Foundation–funded project to further develop analytical methods and to establish reference values for micronutrients in human milk (which do not currently exist) using examples from well-nourished infant-mother pairs in Banjul, The Gambia; Copenhagen; Rio de Janeiro; and Dhaka, Bangladesh. "The quality of the information used to set recommended micronutrient intakes for lactating women, infants, and children will improve greatly once we do this study. And it will be possible to define whether milk micronutrient concentrations in a population group are low such that the mother's or infant's intake needs to be improved," says Allen.

Today's nutrition models. "Our nutrition models now are based on diets that reflect how people actually eat. Current dietary guidelines are based primarily on published studies, but their metabolic effects are rarely tested in people. We do analyses based on a mixed menu for some weeks and in response to a single meal, and can rapidly detect changes in thousands of metabolites in the blood. This is part of nutritional phenotyping; we are trying to figure out how and why different people respond differently to food interventions. As part of the Agricultural Research Service, the research arm of the USDA, we do these interventions that can then lead to dietary recommendations for prevention of chronic disease and support of health."

A global issue. "Micronutrient deficiency is a global issue. In the U.S., our flour is fortified with folic acid, which is turned into folate in the body, to reduce the risk of neural tube birth defects in women who are genetically prone to having offspring with this problem. Folate deficiency often tends to be more prevalent in higher-income countries such as the U.S., Canada, and Scandinavian countries because our diets don't contain enough of the legumes and vegetables that are staple sources of folate in other countries. Milk is fortified with vitamin D in the U.S., and iron is also added to flour."

It takes a village. "In all of our fieldwork, we always had great collaboration with the local investigators, and over time we built a terrific network of international graduate students and postdocs who are now leaders in their own countries. In all our international studies, the local researchers direct the field studies, not us. You cannot do international studies without local support." ■

SCIENTIST TO WATCH

Amélie Gaudin: Data Farmer

Assistant Professor, Department of Plant Sciences
University of California, Davis. Age: 34

BY CATHERINE OFFORD

Having grown up on a farm in France, Amélie Gaudin says it's little wonder she ended up working with crops. "I've been interested in agriculture for a long time," she says. "So I've been following my passion." But it was her experience studying the effects of drought in the early 2000s at the International Potato Center in Lima that gave Gaudin the impetus to pursue this passion academically.

"We farm under the assumption that resources will always be available," she says. But seeing resources under threat from climate change in Peru, "that's when it began to make sense to me that we need to start thinking about farming in a different way."

Keen to explore these ideas, Gaudin joined crop researcher Manish Raizada's lab at the University of Guelph in Ontario, Canada, as a master's student in 2007. There, she undertook a detailed study of the mechanisms underlying responses to low-nitrogen conditions in maize roots. "Phenotyping roots, especially roots of a large plant like maize, is very difficult," says Raizada. So Gaudin proposed an unconventional technique: aeroponics, which grows plants in air misted with nutrient solution. "I didn't know what aeroponics was," Raizada says. "But Amélie went to a hardware store, found some parts, and built the whole system in a greenhouse. It was clear she was going to be a standout."

Gaudin showed that, unlike its wild ancestor, domesticated maize is apparently unable to mitigate nitrogen stress by altering the number of new shoots it produces—instead, it reduces leaf size.[1] "We've been breeding crops so they grow well under high levels of nitrogen fertilizer," says Gaudin. "But we've unconsciously selected for genotypes that are less plastic in response to heterogeneous soil resources."

After earning not a master's but a PhD in 2011, Gaudin did a one-year postdoc with the International Rice Research Institute in the Philippines. In 2012, she returned to Guelph for another postdoc, this time with agroecologist Bill Deen. "She's got a very diverse background," notes Deen, adding that although "the whole area of agroecology was somewhat new to her, she contributed very much to the group's work."

Gaudin analyzed a 31-year data set of crop yields, and in 2015 published a paper with the lab showing that diversity in crop rotation—planting different cereals or legumes between crops—significantly increased maize and soybean yields in arid conditions, and reduced the risk of crop failure.[2] "It was really the first paper that quantitatively demonstrated the resiliency benefit of diversifying rotation," notes Deen. A follow-up published just months later showed that growing soybean and maize in rotation with wheat boosted crop productivity in low-nitrogen soil.[3]

By the end of 2015, Gaudin had established her own lab at the University of California, Davis, where she now studies environmental stress and root ecology. Her program received a funding boost last year from the Foundation for Food and Agriculture Research, which named her an inaugural recipient of its "New Innovator in Food and Agriculture Research" award.

But motivation comes largely from working with science's next generation to tackle future agricultural challenges, says Gaudin—who has supervised trainee scientists since grad school. "She was and is an amazing mentor," Raizada notes. "I always knew her students were going to be inspired." ■

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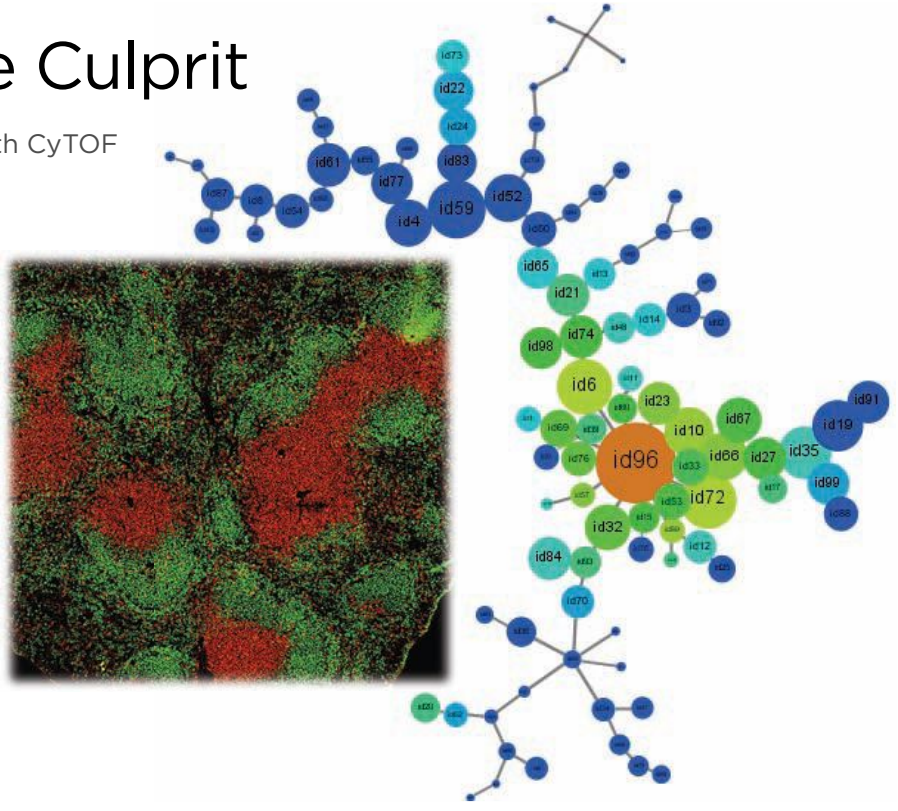
PHOTO BY MAYUMI ACOSTA PHOTOGRAPHY

Pinpointing the Culprit

Identifying immune cell subsets with CyTOF

BY RACHEL BERKOWITZ



MAPPING THE NEIGHBORHOOD: Mouse spleen section stained with 30 antibodies, but showing only two at a time (left). The schematic on the right uses all 30 antibodies to determine cell types and cell neighborhoods. Using tissue sections, immunologists can consider cell types in the context of the tissues and neighborhoods in which they reside.

COURTESY GARRY NOLAN

It was a good day for immunology when Stanford University's Garry Nolan met Scott Tanner at a conference in 2009. Tanner's University of Toronto lab had developed an instrument that could simultaneously measure the expression of 40–50 unique markers in individual cells of a blood or tissue sample.

The tool was Cytometry by Time of Flight, or "mass cytometry" (see Flow Cytometry for the Masses, *The Scientist*, December 2011). As part of this technique, which was a 2011 Top 10 Innovation winner in *The Scientist*'s annual competition, antibodies attached to heavy metal isotopes bind to target antigens in a blood or tissue sample. The cells are sprayed into an argon plasma, or high-temperature ionized gas, which vaporizes them and ionizes the metals. A mass spectrometer analyzes the resulting metal ions, revealing which markers appeared within or on the surface each cell in the sample. Algorithms then group the cells according to antigen expression.

Cytometry by Time of Flight, or CyTOF as it has come to be known, could be used for general pathology or disease diagnostics, but Nolan immediately grasped its power for studying the immune system, which is characterized by a multitude of subtle differences in cell types. In a seminal 2011 paper, Nolan and his colleagues measured 34 proteins and other parameters in single cells from human bone marrow and, by triggering known pathways, identified the phenotype and function of each cell (*Science*, 332:687-96).

Mass cytometry can detect three or more times as many unique markers as conventional flow cytometry, which caps at 10–15. Still, nothing's perfect. Whereas conventional analysis can examine 20,000 cells per second, CyTOF, which is marketed by Fluidigm for ~$650K, is currently limited to 1,000 cells/second. And cells are vaporized in the process, so the original sample cannot be further analyzed. Comparing it to Star Trek's universal medical diagnostic tool, Nolan notes that "Until we have Dr. McCoy's tricorder, we've got to make the most of what we've got."

To explore what CyTOF can teach us about the immune system, *The Scientist* interviewed researchers who are using this new technology to understand immune signals associated with several perplexing diseases.

PROBING LEUKEMIA'S RESPONSE TO TREATMENT

RESEARCHER: Greg Behbehani, Assistant Professor of Internal Medicine, Ohio State University Wexner Medical Center

PROJECT: Some 70 percent of patients who achieve remission following treatment for acute myeloid leukemia (AML) eventually relapse, in part due to a subpopulation of immune cells that becomes resistant to chemotherapy. Behbehani studies how this resistance develops.

APPROACH: Most AML drugs either kill immature cells or block signals that prevent the cells from maturing. Behbehani's lab uses CyTOF to home in on subgroups of AML cells with a phenotype that renders them resistant to a drug. A key component of accomplishing that goal is developing antibody panels capable of revealing cell-cycle markers that indicate a cell's level of maturation, as well as markers to which a drug of interest binds.

At least a hundred different genetic mutations are associated with AML, and these mutations can occur in thousands of different combinations. CyTOF can identify the wide range of functional phenotypic properties that these mutations generate, and can suggest how to target an individual's leukemia cells. One person may need a chemotherapy agent to kill fast-growing cells, while another might benefit most from a signal inhibitor that allows the leu-

kemia cells to grow up and die on their own. Behbehani recently demonstrated that most immature cell populations had very different cell cycle and intracellular signaling properties across AML subtypes. (*Cancer Discov*, 5:988-1003, 2015).

TIPS: Each set of antibodies used for a CyTOF run must be carefully selected, then tagged with metals (if you're not purchasing a predesigned panel); the cells must be incubated with the metal-tagged antibodies, and the signal tested against a reference before it's used in unknown samples. That's a time-consuming process. "If you don't need to look at 30 things simultaneously, it's probably easier to do regular fluorescent flow cytometry," Behbehani says.

He also advises new users to do careful controls, as a missed control is often the culprit behind data full of artifacts or doublets, which occur when ion clouds fuse with each other during vaporization. The best strategy for managing doublets is to reduce the rate at which cells are sampled during CyTOF, and to label antibodies with combinations of metals, such that a doublet would appear as a combination that can't occur on its own.

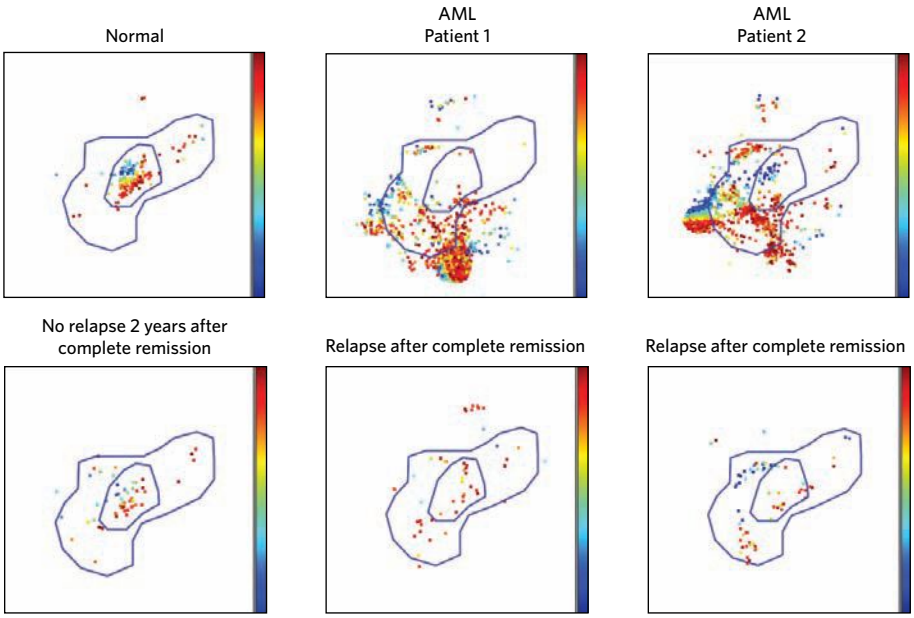
LOOKING FORWARD: As new drugs for AML are tested in clinical trials, Behbehani plans to use CyTOF to identify subpopulations of immune cells affected by these compounds. The idea is to determine how drugs work at the single-cell level, and to better match a particular patient to his or her most effective therapy option.

RESPONDING TO CANCER AND INFECTION

RESEARCHER: Evan Newell, Principal Investigator, Singapore Immunology Network, Agency for Science, Technology and Research

PROJECT: Newell's lab aims to identify and profile antigen-specific T cells that respond to cancers and infectious diseases such as hepatitis B virus (HBV), with the goal of developing immunotherapies that can modulate these cells' responses.

APPROACH: Newell's lab uses CyTOF to look for candidate T cells involved in HBV or cancer, but with a twist: the researchers add a cocktail of so-called model antigens to their antibody panels in order to boost the number of markers they can track.



CLASSIFYING CELLS: (Top panels) Analyses of a subset of immune cells using CyTOF labeling and separation illustrates a normal phenotype pattern (blue outlines) compared to that of two patients with acute myeloid leukemia (AML). (Bottom panels) Patients with complete remission and no relapse show normal immunophenotypic patterns compared to patients who have relapsed after remission. Cells expressing no CD38 are colored blue, while progression to red indicates increasing amounts of the cell-surface marker.

COURTESY GREGORY BEHBEHANI

Model antigens are peptides combined with other molecules known to activate T cells in response to foreign antigens. Called peptide-MHC tetramers, these laboratory-synthesized structures are also labeled with unique combinations of metal isotopes. They bind to yet another type of T-cell surface molecule that recognizes fragments of an antigen. This multiplexing technique provides the opportunity to identify and characterize, at a single-cell level, hundreds of different T-cell specificities in one CyTOF run. The team is currently screening more than 500 possible antigens specific to HBV (*Nature Biotechnol,* 31:623-29, 2013).

COMMON RESOURCE: Newell and colleague Michael Wong, now at Merck, have also created an atlas of T-cell types from 8 different human tissues, charting out cells that are found across tissues and those that are unique to each. They are now studying how antigen specificity of these cells leads to diverse profiles in different tissues (*Immunity,* 16:442-56, 2016).

CHALLENGES: Cell preparation and data-gathering rates in mass cytometry are less efficient than in flow cytometry, although the techniques are improving. "Often, I have to perform a 15–18 hours nonstop acquisition for my precious patient samples," says Yang Cheng, a graduate student in Newell's lab. The same samples can be processed in 4 hours using flow cytometry.

Visualizing and understanding these large, multidimensional data sets also poses an analytical challenge. To help solve this problem, Newell published an algorithm that helps to distinguish T-cell diversity according to user-defined categories, which include different markers specific to functionality and markers that indicate stages of T-cell differentiation (*J Immunol,* 196:924-32, 2016). "The awesome thing about this algorithm is that it's not only applicable to mass cytometry data, but [we've] also used [it] to analyze NBA basketball statistics," says Wong.

LOOKING FORWARD: Newell's lab is investigating the markers expressed on virus-specific "killer" T cells at different clinical stages of cancer and/or viral infection. He's also spun off a company called immunoSCAPE that characterizes mouse and human immune-cell subsets in blood and tissue samples on a fee-for-service basis.

DECONSTRUCTING RHEUMATOID ARTHRITIS

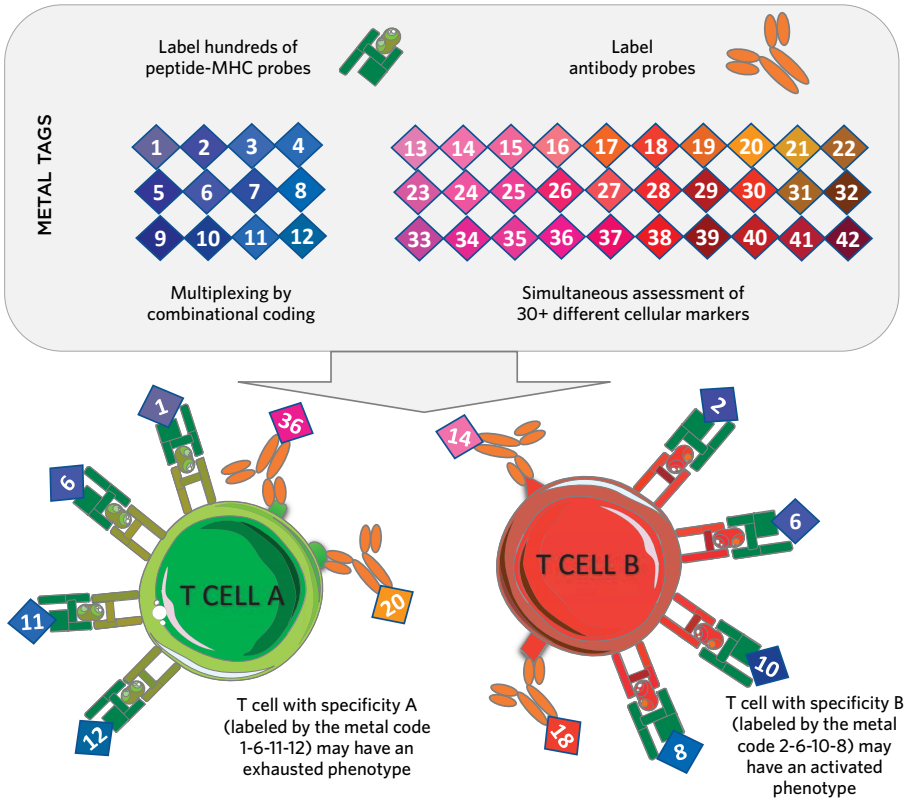
RESEARCHER: Michael Brenner, Principal Investigator, and Deepak Rao, Instructor in Medicine, Brigham and Women's Hospital, Boston

PROJECT: Brenner and Rao seek to identify specific types of immune cells that cause rheumatoid arthritis, an autoimmune disease in which the immune system attacks the synovial membrane that lines joints, as well as other tissues. Ultimately, their goal is to identify abnormal immune pathways and to understand how to manipulate the signals that regulate them.

APPROACH: The researchers used CyTOF with a cocktail of 35 different antibodies to recognize the proteins on individual cells, and thus identify the subgroups of immune cells that infiltrate the joints of people with the condition. They found that a quarter of all $CD4^+$ T helper cells, which regulate the activity of all other immune cells, had exceptionally high expression of programmed cell death protein 1 (PD-1), a cell-surface receptor that acts to tamp down T-cell activity and promote self tolerance (*Nature,* 542:110-14, 2017).

To figure out what these PD-1–marked T cells were doing, Rao and Brenner stained another sample with

SINGLE-CELL ASSESSMENT OF T-CELL SPECIFICITY, PHENOTYPE, AND FUNCTION



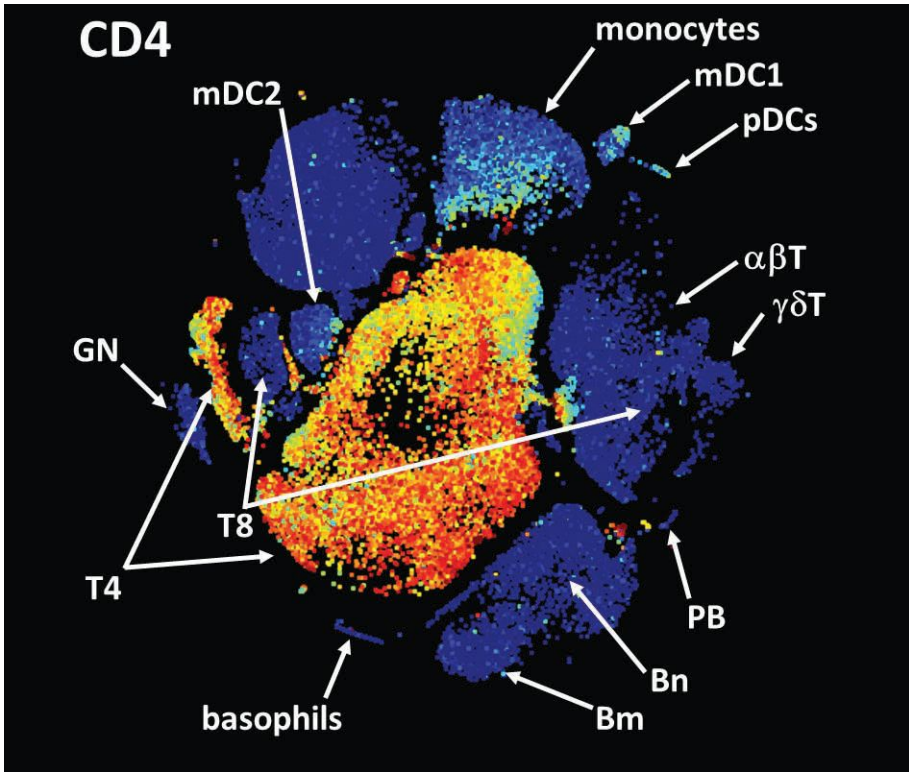
ACTIVITY PROBING: Peptide-MHC probes and antibody probes are labelled with combinations of metal tags. These bind to T cells with different specificities (A or B) that may have exhausted or activated phenotypes. The novel multiplexing technology allows for probing of extensive candidate targets in a single sample.

COURTESY EVAN NEWELL

just the antibodies that had been found attached to the subgroups present in the tissues, and conducted gene expression profiling in these cells. Doing so revealed that the PD-1–enhanced T cells in the patient's joints are in fact still functioning—and indeed, are stimulating B cells to produce antibodies within the inflamed joint tissue, effectively fueling inflammation.

LIMITATIONS: CyTOF triples the number of markers that can be measured on one cell in a single run compared to conventional techniques, but it requires a relatively large sample size, approximately 50,000 cells. In some contexts, this can be a problem. Rao and Brenner could obtain the needed sample size, but Rao notes that identifying a subpopulation is just the start—CyTOF provides no information on how this subpopulation arises. To get at these questions, he stains a new sample with the same antibodies and sorts the cells using conventional flow cytometry, to obtain samples for RNA sequencing.

LOOKING FORWARD: Rao and Brenner next plan to develop modified CyTOF antibody panels to home in on other populations of immune cells that regulate how the PD-1–enhanced T cells signal in rheumatoid arthritis. They will also use this approach to explore whether these cells pop up in other autoimmune diseases.



IMMUNOLOGICAL POINTILLISM: Maps of peripheral blood samples from Sjögren's patients. Each dot is a cell; and the color reflects expression of the CD4 marker. The distance between dots reflects overall similarity in the expression of all markers that were measured. (Dendritic cells, DC; granulocytes, GN; CD4 T cells, T4; CD8 T cells, T8; various B cell types, B)

EXPLAINING SJÖGREN'S SYNDROME

RESEARCHER: Michaël Mingueneau, Research Scientist and Group Leader, Biogen; Xavier Mariette, Head of Rheumatology, Bicêtre Hospital, Paris-Sud University

PROJECT: Mingueneau and Mariette are investigating how different immune-cell types contribute to Sjögren's syndrome, an autoimmune disease that causes dryness of the eyes, mouth, and other exocrine glands, as well as many systemic complications including lymphoma.

APPROACH: Previous efforts to characterize Sjögren's focused on a few cell types or subsets. In contrast, Mingueneau and Mariette designed a CyTOF antibody panel encompassing 34 surface markers to detect a broad range of lymphoid and myeloid cell subsets, thus targeting many of the most common types of immune cells. Using this panel, they compared blood and lacrimal gland biopsy samples from 49 individual patients with those of 45 control donors to identify key pathogenic cell types (*J Allergy Clin Immunol,* 137:1809-21, 2016).

They identified multiple immune-cell profiles that had not previously been considered pathogenic in Sjögren's. For example, patients with low numbers of helper T cells and memory B cells, as well as those with high numbers of activated killer T cells, experienced more-severe disease. Mingueneau and Mariette hope to use these results to optimize therapies for individual patients. "This is a first large-scale clinical application of mass cytometry to stratify a patient population with a chronic disease," says Mingueneau.

CHALLENGES: Mingueneau and Mariette have gone a long way towards demonstrating CyTOF's clinical application, but the cost of the machine and the complexity of operating it limit CyTOF's broader adoption for now. Researchers are developing easier-to-use, standardized instrumentation that will be crucial for its use in clinical settings.

LOOKING FORWARD: In addition to potentially obviating the need for repeated tissue biopsy sampling during clinical trials, the researchers hope that their CyTOF analysis can match therapies to individual patients. "One could envision in the future enrolling patients in clinical trials according to their disease biomarker signature," says Mingueneau. ■

COURTESY MICHAËL MINGUENEAU

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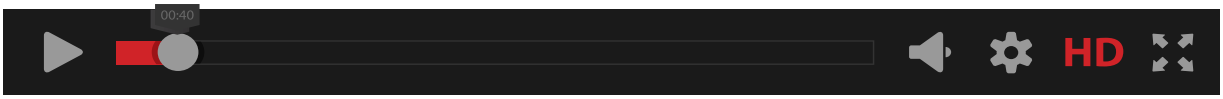

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Lessons from the North

Iceland's unique combination of genetic homogeneity, genealogical tradition, and high participation in research make it a prime location for discovery and validation of drug targets.

BY CATHERINE OFFORD

A few years ago, Brian Durie and his colleagues at the International Myeloma Foundation (IMF) identified a problem. They knew that people who develop multiple myeloma previously had its benign precursor, a condition known as monoclonal gammopathy of undetermined significance (MGUS). But they also knew that most doctors don't routinely screen for this condition, in which plasma B cells harmlessly produce an abnormal peptide called M protein. As a result, most cases are never diagnosed unless patients develop multiple myeloma or related diseases. (Progression to malignancy is not inevitable, but some estimates put the risk as high as nearly 40 percent over a lifetime.)

"To have a chance to cure the disease, you really need to be able to intervene in patients who have early disease," explains Durie, IMF's cofounder and chairman. And because only about 1 percent of people with MGUS will go on to develop the cancer per year, "you need to have very clear criteria for patients who have early disease but are definitely in the process of progressing to myeloma."

Given the low rates of MGUS diagnosis, researchers have struggled to study this transition. But in 2015, after discussions with other scientists working on myeloma, Durie and his colleagues hit upon a potential solution. "We were in touch with researchers basically all around the world," he says. At some point, "it emerged that Iceland could be a very good place to achieve some of our goals."

With a tiny population—only around 320,000 people as of 2013—plus the most detailed national medical and genetic databases of any country in the world, Iceland offered a unique experimental setting. "One of the main advantages of Iceland is that we can detect and document all the people with MGUS," Durie explains. "If, by studying them closely, we can figure out which ones are going to get myeloma, then that gives us the opportunity to intervene precisely, early on, and provide a cure."

Teaming up with University of Iceland epidemiologist Sigurdur Kristinsson, the IMF launched a nationwide MGUS screening study last November. Since then, Kristinsson and his colleagues have been travelling around the country, which reports around 20 new cases of multiple myeloma per year, encouraging all of the roughly 140,000 Icelanders older than 40 years of age to take part. With more than 75,000 people registered and nearly 20,000 blood samples as of mid-April, the project is already the largest myeloma study every conducted. "The process is incredible," says Durie. "It's been going amazingly well."

The project's early progress is unlikely to come as a surprise to researchers already familiar with biomedical research in Iceland,

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however. The island country's unique combination of high participation rates, genealogical tradition, and sophisticated medical infrastructure provide a living lab like no other, allowing scientists studying any number of conditions to connect the dots between genes, environment, and disease. And with the help of the country's biopharmaceutical powerhouse and Amgen subsidiary deCODE Genetics—a trailblazer in exploring human variation over the last two decades—researchers are hoping to turn these resources into effective therapies for people all over the world.

Icelandic sagas, then and now

Iceland was settled by Norwegian and Celtic explorers in the 9th century—many millennia after much of the rest of Europe. The country's population has remained relatively small ever since, thanks to geographic isolation, a harsh environment, and a series of genetic bottlenecks caused by two waves of plague during the 15th century, a spate of smallpox in the early 1700s, and a devastating volcanic eruption in 1783. As a consequence, the island has one of the most genomically homogenous populations in Europe.

"In that setting, where lots of people are very similar genetically, it's easier to pinpoint a variant that's disease-causing in some way," explains Julia Wilson, associate director of the Wellcome Trust Sanger Institute.

But it's not just Iceland's unusual demographics that get researchers excited. The country also has one of the oldest gene-

BANKING ON ICELAND: Researchers at the International Myeloma Foundation in North Hollywood, California (left), in collaboration with the National University Hospital of Iceland, store thousands of blood samples from Icelanders in a collection of freezers (center), testing the samples with automatic blood analyzers (right and far right) for genetic variants that might be linked to the risk of developing of multiple myeloma.

alogical traditions in the world, dating back to the 12th century, when Icelandic priest Ari the Wise traced the country's history, including its family trees, in a tome called Íslendingabók—the book of Icelanders. Since then, church records and censuses have kept track of births, deaths, and marriages, resulting in a detailed and ongoing picture of Icelandic ancestry.

This tradition received a modern makeover in 1996, when Icelandic geneticist Kári Stefánsson founded deCODE Genetics and curated all available genealogical information into a single online database, also called Íslendingabók. Accessible by anyone with an Icelandic social security number, the database proved popular among members of the public as well as scientists. By the mid-2000s, it was recording more than 1,000 log-ins per day, and in 2013, deCODE launched an app allowing users to determine their relatedness to any other Icelander—complete with an "incest alarm" feature.

But the real research power of these genealogical data comes about through integration with two more databases, also assembled by deCODE. One details genetic information collected from biological samples of more than 100,000 citizens; the other stores medical records of people participating in related research projects, collected from national registries. "Every single cancer that is diagnosed in Iceland is centrally registered," Kristinsson explains. "And all medical procedures, clinical diagnoses, prescriptions—they're all registered too."

Of course, such data volumes would be impossible without the participation of Icelanders themselves, and it's here that an additional benefit of Iceland's close-knit community plays the biggest role, with information about health initiatives spreading quickly and contributing to research participation rates that far exceed the rest of Europe. "Everyone here watches a single TV news channel," says Kristinsson, who appeared on the news and at a subsequent press conference with Icelandic president Guðni Jóhannesson to publicize the IMF's work last year. "When something happens here in Iceland, everyone is aware of what's going on."

Northern powerhouse

The last two decades have seen an outpouring of research from Iceland, both documenting variation in the genomes of its inhabitants and laying the groundwork to develop effective therapeutics for genetic disorders. Much of this research has been driven by deCODE Genetics. "We have looked at all kinds of diseases, but also physiological function, hair color, height, weight," says Stefánsson, who serves as deCODE's CEO. "I think the fact that we have gathered very large amounts of data—longitudinally and horizontally—gives us a tremendous advantage, because we have been able to proceed without having too many a priori assumptions."

In 1998, recognizing the potential of deCODE's approach for the discovery of novel drug targets, Basel, Switzerland–based Hoffman-La Roche poured $200 million into the fledgling company with the aim of developing gene-based therapies for 12 common disorders. And in the early 2000s, deCODE invested in its own diagnostics and drug development, even advancing one therapeutic for cardiovascular disease—based on company researchers' discovery of genes associated with heart attack risk—as far as Phase 3 trials.

> In that setting, where lots of people are very similar genetically, it's easier to pinpoint a variant that's disease-causing in some way.
>
> —Julia Wilson, Wellcome Trust Sanger Institute

The journey has not been without fits and starts, however. In 2006, the company suspended its heart drug trial over efficacy concerns, and began working to improve the formulation of the tablets being given to patients. But shortly after the 2008 financial crisis, deCODE was forced to declare bankruptcy, and the study was never relaunched. In the following years, the company suspended its drug-development programs altogether and backed away from providing personalized genetic tests. In 2012, U.S.-based Amgen acquired deCODE for $415 million in cash.

Since then, the Icelandic portion of Amgen's research has generated a constant stream of high-profile genetic discoveries as well as new diagnostics and therapeutics. One of deCODE's key commercial findings was a genetic variant in a handful of Icelanders that disrupted the BACE1 enzyme pathway and conferred lower risk of Alzheimer's—confirming the protein as a viable drug target (*Nature*, 488:96-99, 2012). "The mutation carries almost complete protection against Alzheimer's," says Stefánsson. "It basically showed the industry: if you succeed in making a good inhibitor, you will slow down and even prevent Alzheimer's disease."

ALL PHOTOS COURTESY OF IMF

And just last year, Amgen announced that deCODE had identified a loss-of-function variant of the *ASGR1* gene that reduces the risk of heart attack by nearly 35 percent (*New Engl J Med*, 374:2131-41, 2016). Amgen is already developing an inhibitor for the gene product, and the company's chief of R&D Sean Harper told *Forbes* shortly after the discovery that, barring major mishaps, the team was hoping to get a candidate drug to the clinical testing stage within two years.

DeCODE researchers regularly collaborate with numerous Icelandic research organizations, such as the Icelandic Heart Association and Landspítali University Hospital in Reykjavik. Earlier this year, an Icelandic team published the results from an analysis of whole-genome sequence data from more than 15,000 Icelanders, identifying large-scale diversity in DNA sequences missing from the human reference genome, as well as another genetic variant linked to risk of heart attack (*Nat Gen*, 49:588-93, 2017). And in 2015, deCODE researchers and collaborators all over Europe published four papers in a special issue of *Nature Genetics* describing, among other things, mutations in *ABCA7* associated with increased Alzheimer's risk and a rare genetic variant of *MYL4* that predicts early-onset atrial fibrillation.

As with deCODE's previous findings, these studies open new avenues for pharmaceutical companies looking for ways to understand and target disease. "There are many such examples showing how working on genetics can empower the development of new drugs," says Stefánsson, adding that "there are probably 100 diseases we aspire to make a contribution to before the end of this year."

Beyond Iceland

With such an outpouring of biomedical results, it's little wonder that some organizations have attempted to replicate Iceland's research recipe elsewhere. As early as the 2000s, genetic pioneer Craig Venter reportedly tried to launch a company modeled on deCODE to sequence the genomes of the Saudi Arabian population, though the project fell through due to privacy concerns surrounding the commercial export of DNA data from the country.

More recently, Qatar began a similarly ambitious sequencing program, with organizers mentioning similarities with deCODE's research, thanks to comparable population sizes and homogenous ancestry. And Genomics Medicine Ireland—a Dublin-based startup supported by investments from Amgen—had raised more than $40 million by last November to launch its own deCODE-inspired program to develop diagnostics and drugs informed by sequencing Irish DNA.

But despite the advantages of the Icelandic approach, there are also limitations associated with the very features that make it so informative—particularly when it comes to testing the gene-based therapies themselves. "I'm not sure that Iceland is a particularly good place to conduct clinical trials," says Stefánsson. "You usually want to conduct trials in a relatively heterogeneous population so you can make a claim that the beneficial effect of the drug you're trying is not specific to a particular population."

"You're looking at a large cohort of very similar people," agrees the Sanger Institute's Wilson. "It means that findings you get may not extrapolate to a sub-Saharan African population, or a Bengali population, for example." What's more, Iceland's small population might simply not provide enough data, she adds. "Particularly for complex diseases, such as bowel disease, where multiple genes are involved, you need to be analyzing huge data sets to pinpoint variants."

Nevertheless, Durie argues that the results generated in Iceland will inform research into human genomic variation around the world. In the IMF's work on myeloma, for example, "it's difficult to replicate these studies elsewhere," says Durie. "But I think what's more important is that the information that's gathered in Iceland can be extrapolated, and then applied globally. It's a unique testing environment in which we can gather information we can then use everywhere."

For most Icelanders, this is a responsibility borne with pride, says IMF study patron and former Icelandic president Vigdís Finnbogadóttir, who has been a lifelong supporter of the country's progress in medical research. "I'm extremely proud," the octogenarian tells *The Scientist*. "And I think if Icelanders can contribute to the health of the world, I'm more than proud. I'm grateful." ■

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The Yuck Factor

The human brain's insular cortex is adept at registering distaste for everything from rotten fruit to unfamiliar cultures.

BY ROBERT SAPOLSKY

Considerable human misery turns out to be attributable to an obscure corner of the brain, the insula.

In most mammalian brains, the insula's function is straightforward. If a rat, say, bites into rancid food, its insula protects it from being poisoned by triggering nausea and gagging. In humans, however, the insula is not merely about gustatory disgust. If someone recounts something "rotten" they once did, or hears about someone else's similar behavior, their insula activates. In other words, the insula also processes moral disgust. For example, if someone backstabs you in a game, the magnitude of your insula's activation predicts how outraged you'll feel, and how vengeful you'll act. Our insula responds mainly to the disgustingness of sentient, intentional harm—if the person stabbed in the back believes a computer was to blame, her insula remains quiet. And if we're sufficiently morally disgusted, we even feel sick to our stomachs. Our brains fail to distinguish between literal disgust at a fetid taste and metaphorical disgust at a rotten act. I touch on this neurobiological quirk in my latest book, *Behave*.

This intertwining of the literal and metaphorical reflects evolutionary tinkering, not inventing. When hominins first developed this capacity for morality, it wasn't driven by a new brain region. Instead, evolution duct-taped moral disgust onto the insula's existing repertoire, and the region occasionally confuses it with gustatory disgust.

This intertwining certainly has an upside. Righting moral wrongs can demand great sacrifice, and it would be hard to work up a head of steam for that if moral transgressions were mere detached abstractions. A stomach churning with outrage can supply the visceral fuel that makes moral imperatives feverishly vital.

But there are downsides. It's tempting to make disgust a litmus test for deciding what's wrong—if it makes you puke, then you must rebuke. But one person's repugnance is another's loving lifestyle. Moral disgust is mired in time, place, and parochialism.

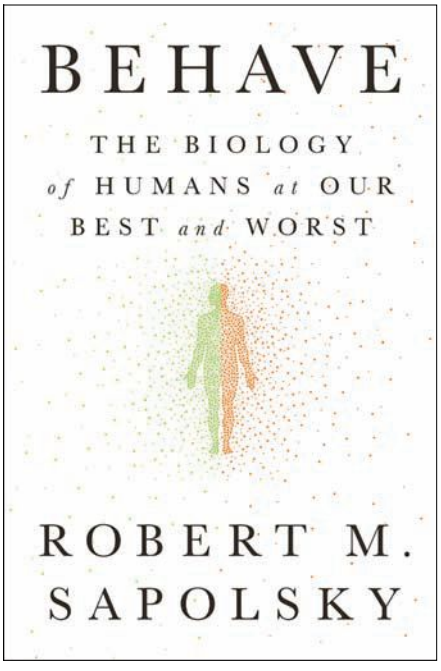
A stomach churning with moral outrage can also invite hatred, equating "different" with "deplorable," deciding that *They* think, live, love, and pray abnormally.

All despots know this, and rely on activating their minions' insulas by making the objects of their personal outrage into something more broadly disgusting: drug smugglers and rapists; blasphemers and infidels; vermin and malignancies.

There's another danger. It's hard to meet a stranger and know if they share your deepest values. But if those values are yoked to a marker, such as style of dress, the task becomes easier. Know nothing more about two people than that one wears a Stetson and the other a sari, and you can confidently guess who of the two eats cows versus worships them.

This provides a dangerous stepping-stone. Being disgusted by someone's abstract beliefs is tough for an insula. But feeling disgusted by others because, say, they eat repulsive, sacred, or adorable animals—this the insula can sink its teeth into. This impulse supplies the momentum to decide that, as long as you're at it, *They* also have disgusting ideas about, say, deontological ethics.

If a subordinate baboon is brutalized by a dominant thug, a third baboon may be galvanized into protecting the victim (especially if they are related). But only a human would fight the unjust treatment of a distant stranger, an endangered species, or a fragile ecosystem. My guess is that this only occurs because of the human insula's uniqueness: the fact that an injustice can make our innards blaze with a need to act. But that same blazing leads so many to grab pitchforks and join the mob. Innovation can be cool—like controlling fire, Snapchat filters, or the insula becoming a multitasker. But innovation usually turns out to be a double-edged sword. ■



Penguin Press, May 2017

Robert Sapolsky is a neuroendocrinologist at Stanford University and the author of several books. Read an excerpt of Behave: The Biology of Humans at Our Best and Worst *at www.the-scientist.com.*

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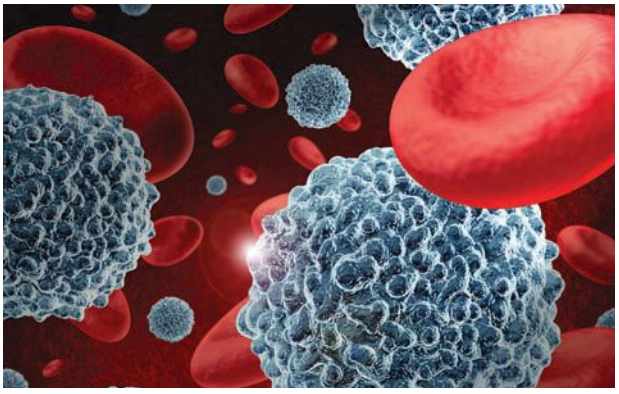

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Cold Spring Harbor Laboratory Meetings & Courses
2017 Late Summer & Fall

www.cshl.edu/meetings

Cold Spring Harbor Laboratory on a warm October morning

Meetings

Genome Engineering: The CRISPR/Cas Revolution
July 21 - 24 Jennifer Doudna, Maria Jasin, Stanley Lei Qi, Jonathan Weissman

Cell Death
August 15 - 19
David Andrews, Anthony Letai, Karen Vousden

Eukaryotic mRNA Processing
August 22 - 26 Jean Beggs, Alberto Kornblihtt, Jens Lykke-Andersen

Mechanisms of Eukaryotic Transcription
August 29 - September 2
Patrick Cramer, John Lis, Jane Mellor

Eukaryotic DNA Replication & Genome Maintenance
September 5 - 9 Karlene Cimprich, Anne Donaldson, Anindya Dutta

Microbial Pathogenesis and Host Response
September 12 - 16 Denise Monack, Raphael Valdivia, Malcolm Whiteway

Annexins
September 17 - 20 Katherine Hajjar, Jacob Rand, Laura Santambrogio

Stem Cell Biology
September 25 - 29
Fiona Watt, Marius Wernig, Ken Zaret

Neurobiology of *Drosophila*
October 3 - 7
Heather Broihier, J. Troy Littleton

Biology of Cancer: Microenvironment & Metastasis
October 10 - 14 Scott Lowe, Senthil Muthuswamy, M. Celeste Simon

Next Generation Cancer Clinical Trials
October 13 - 15 Elizabeth Jaffee, Robert Maki, David Tuveson

Forty Years of RNA Splicing: From Discovery to Therapeutics
October 22 - 25
Mila Pollock, Phillip Sharp, Joan Steitz

Genome Informatics
November 1 - 4 Janet Kelso, Aaron Quinlan, Melissa Wilson Sayres

Single Cell Analyses
November 8 - 11
Nancy Allbritton, Scott Fraser, Junhyong Kim

STATs: Importance in Basic & Clinical Cancer Research
November 15 - 18 James Darnell, David Levy, Valeria Poli, George Stark

Plant Genomes & Biotechnology From Genes to Networks:
November 29 - December 2 David Jackson, Todd Mockler, Jane Parker, Sue Rhee

Development and 3-D Modeling of the Human Brain
December 6 - 9
Paola Arlotta, Sergiu Pasca

Courses

Programming for Biology
October 16 - 31
Simon Prochnik, Sofia Robb

X-Ray Methods in Structural Biology
October 16 - 31 William Furey, Gary Gilliland, Alexander McPherson, Anastassis Perrakis, James Pflugrath

Antibody Engineering, Phage Display & Immune Repertoire Analysis
October 17 - 30
Don Siegel

Advanced Sequencing Technologies & Applications
November 7 - 18
Malachi Griffith, Obi Griffith, Elaine Mardis, Richard McCombie, Aaron Quinlan

Cryo-Electron Microscopy
November 7 - 20
Justin Kollman, Gabriel Lander, Melanie Ohi, David Veesler

Foundations of Computational Genomics
November 29 - December 6
Aaron Mackey, William Pearson, Lisa Stubbs, James Taylor

Immersive Approaches to Biological Data Visualization
December 7 - 16
Kelly Gaither, Matthew Vaughn

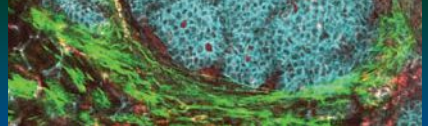
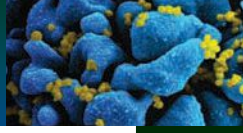
The Genome Access Course
November 13 - 15
Assaf Gordon, Emily Hodges, Benjamin King, Steven Munger

Professional Development:

Scientific Writing Retreat
November 15 - 19
Charla Lambert, Stephen Matheson

Cold Spring Harbor Laboratory, Meetings & Courses Program, Cold Spring Harbor, New York 11724 USA



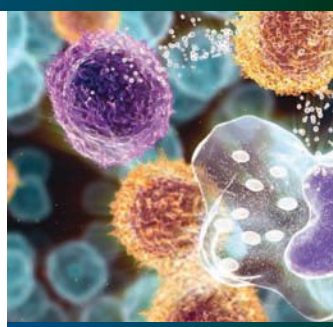



Registration Open for Keystone Symposia's 60 Conferences

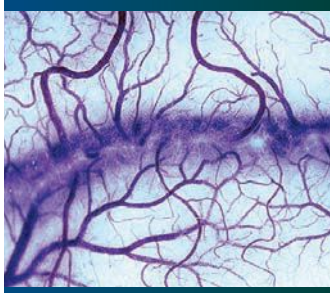
September 2017– June 2018

Vectors, Pathogens & Diseases: Current Trends & Emerging Challenges (T1)
Maternal-Fetal Cross Talk: Harmony vs. Conflict (T2)
Regenerative Biology & Applications: Cell Differentiation, Tissue Organization & Biomedical Engineering (T3)
Antimicrobials & Resistance: Opportunities & Challenges (T4)
Frontiers of Serotonin Beyond the Brain (T5)
Heart Failure: Crossing the Translational Divide (A1)
State of the Brain: Genetic Dissection of Brain Circuits & Behavior in Health & Disease (A2)
T Cell Dysfunction, Cancer & Infection (A3)
Plant Signaling: Molecular Pathways & Network Integration (A4)
Natural Products & Synthetic Biology: Parts & Pathways (J1)
Tumor Metabolism (A5)
Cell Death, Inflammation & Adaptation to Tissue Stress (A6)
Organ Crosstalk in Obesity & NAFLD (J3) *joint with* **Bioenergetics & Metabolic Disease (J4)**
DNA & RNA Methylation (A7) *joint with* **Ubiquitin Signaling (A8)**
Translational Systems Immunology (A9)
Precision Genome Editing with Programmable Nucleases (B1)
Emerging Technologies in Vaccine Discovery & Development (J5) *joint with* **Progress & Pathways Toward an Effective HIV Vaccine (J6)**
Atherosclerosis: Lessons Learned & Concepts Challenged (B2)
Frontiers in Islet Biology & Diabetes (B3)
Cryo-EM from Cells to Molecules: Multi-Scale Visualization of Biological Systems (F1)
Cancer Epigenetics: New Mechanisms, New Therapies (B4)
Phosphoinositide Biology: New Therapeutic Targets Beyond Class I PI3K (B5)
Emerging Cellular Therapies: T Cells & Beyond (B6) *joint with* **Lymphocytes & their Roles in Cancer (R1)**
Mobile Genetic Elements & Genome Plasticity (B7)
GPCR Structure & Function: Taking GPCR Drug Development & Discovery to the Next Level (B8)
Regulation & Dysregulation of Innate Immunity in Disease (B9)
Antibodies as Drugs: Translating Molecules into Treatments (C1)
Noncoding RNAs: Form, Function, Physiology (C2)
Endoderm Development & Disease: Cross-Organ Comparison & Interplay (C3)
Uncomplicating Diabetes: Reducing the Burden of Diabetes-Related End-Organ Injury (J7) *joint with* **Vascular Biology & Human Diseases: From Molecular Pathways to Novel Therapeutics (J8)**
Immunological Memory: Innate, Adaptive & Beyond (X1) *joint with* **Aging, Inflammation & Immunity (X2)**
Manipulation of the Gut Microbiota for Metabolic Health (X3) *joint with* **Microbiome, Host Resistance & Diseases (X4)**
Cells vs. Pathogens: Intrinsic Defenses & Counterdefenses (C4)
Cancer Immunotherapy: Combinations (C5)
Chromatin Architecture & Chromosome Organization (X5) *joint with* **Gene Control in Development & Disease (X6)**
The Resolution of Inflammation in Health & Disease (C6)
iPSCs: A Decade of Progress & Beyond (C7)
Organs- & Tissues-on-Chips (D1)
Myeloid Cells (D2)
Therapeutic Targeting of Hypoxia-Sensitive Pathways (V1)
Pushing the Limits to Healthspan & Longevity (D3)
Tuberculosis: Translating Scientific Findings for Clinical & Public Health Impact (X7) *joint with* **HIV & Co-Infections: Pathogenesis, Inflammation & Persistence (X8)**
Mitochondrial Biology (Z1) *joint with* **Selective Autophagy (Z2)**
Precision Medicine in Cancer (E1)
Exosomes/Microvesicles: Heterogeneity, Biogenesis, Function & Therapeutic Developments (E2)
One Million Genomes: From Discovery to Health (G1)
Novel Aspects of Bone Biology (E3)
B Cells: Mechanisms in Immunity & Autoimmunity (E4)
Advances in Neurodegenerative Disease Research & Therapy (Z3) *joint with* **New Frontiers in Neuroinflammation: What Happens When CNS & Periphery Meet? (Z4)**

7 NEW TOPICS



10 NOVEL JOINT PAIRINGS



25 VENUES GLOBALLY

Conferences listed in chronological order. View details for each at **keystonesymposia.org** followed by a **/18** & the alpha-numeric **program code** (e.g., **www.keystonesymposia/18A1**). Registered attendees of one meeting in a joint pair may attend sessions of the other at no additional cost, pending space availability, and can take advantage of the joint poster sessions and social breaks.

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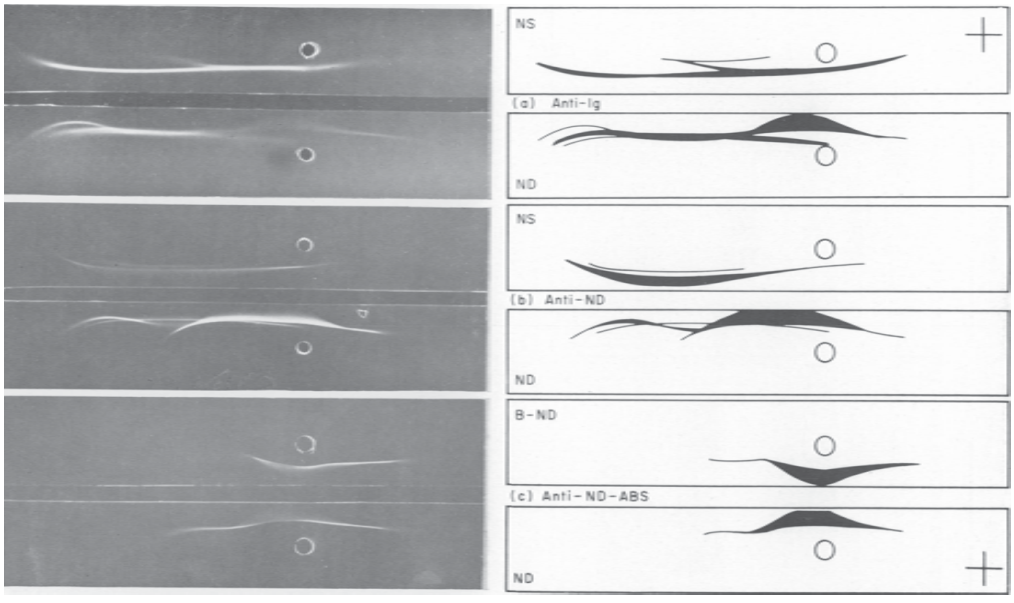
The Discovery of IgE, 1960s

BY ANDREA ANDERSON

In the early 1960s, Kimishige Ishizaka, then an immunologist at the Children's Asthma Research Institute and Hospital in Denver, volunteered himself as a human pincushion. In pursuit of understanding what was then a mysterious protein called reagin, Ishizaka had colleagues inject solutions of the protein into his own back. His self-torture—and that of peers around the globe who would likewise offer up their own skin for experiments—would ultimately lead to the discovery of immunoglobulin E (IgE), an antibody responsible for allergic reactions.



IN PURSUIT OF IgE: University of Uppsala researchers Gunnar Johansson and Hans Bennich characterized a protein from a myeloma patient using electrophoresis (drawings to the right reflect results in corresponding gels). The duo saw massive amounts of immunoglobulin (a) in the patient's serum (ND), eclipsing that in normal serum (NS). Antibodies raised against the ND protein produced markedly similar patterns (b), suggesting ND was an immunoglobulin—a notion supported by removing ND light chains before running the gel (c). The remaining heavy chains belonged to IgE.

Researchers had been collecting clues about reagin—a molecule implicated in hay fever, allergic asthma, and other allergic conditions—decades before Ishizaka's self-experimentation. Inspired by a 1919 report of a new horse allergy in a patient who received blood from a horse-sensitive donor, German researcher Carl Prausnitz injected serum from a fish-allergic colleague, Heinz Küstner, into his own arm in 1921.

The following day, Prausnitz pumped fish extract into the same site and the allergy emerged, a bright-red rash on his arm. With that, Prausnitz launched the Prausnitz-Küstner (P-K) test, one of several key tools ultimately used to discover IgE.

But before IgE was recognized, Ishizaka, with his wife and collaborator Teruko Ishizaka and colleague Margaret Hornbrook, took a detour. In 1963, they reported that reagin was a new form of immunoglobulin A (IgA). Soon after, they had doubts. For one thing, researchers had trouble reproducing the results. The University of Birmingham's Dennis Stanworth later wrote that he also "hotly contested" the idea that reagin was IgA when the Ishizakas visited his lab in the 1960s—a move he believes "encouraged them to look again at this question."

In 1966, the Ishizakas and Hornbrook used the P-K test to show persistent ragweed sensitivity in serum from affected individuals, even after adding antibodies to slurp up IgA (*J Immunol*, 97:75-85). The rash could be avoided by tossing in antibodies raised against reagin in rabbits, though, as the experiment on Ishizaka's back had revealed.

Producing antibodies to reagin "was actually the key," suggests Toshiaki Kawakami, an immunology researcher at the La Jolla Institute for Allergy and Immunology, where Kimishige Ishizaka was the founding scientific director and former president. "Using that antibody, they could identify that the factor was a new molecule because of the size and because of the activity," says Kawakami.

The Ishizakas were not alone in their pursuit of reagin. At the University of Uppsala in Sweden, Gunnar Johansson and Hans Bennich were intently unraveling crucial physical and chemical properties of IgND, a protein found at sky-high levels in blood from an individual (initials N.D.) with an unusual form of myeloma. The duo, along with Stanworth and John Humphrey in the U.K., went on to show (in Humphrey's skin) that, when delivered first, IgND blocked reagin's activity, presumably because IgND was already interacting with reagin's targets (*The Lancet*, 290:330-32, 1967).

Suspecting they were working on the same molecule, the Johansson- and Ishizaka-led teams swapped reagents and confirmed reagin and IgND were the same protein and a new form of immunoglobulin. In 1968 they agreed upon naming it IgE (*Immunology*, 15:323-24). ■

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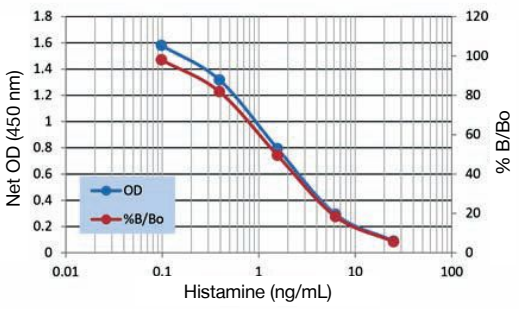

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