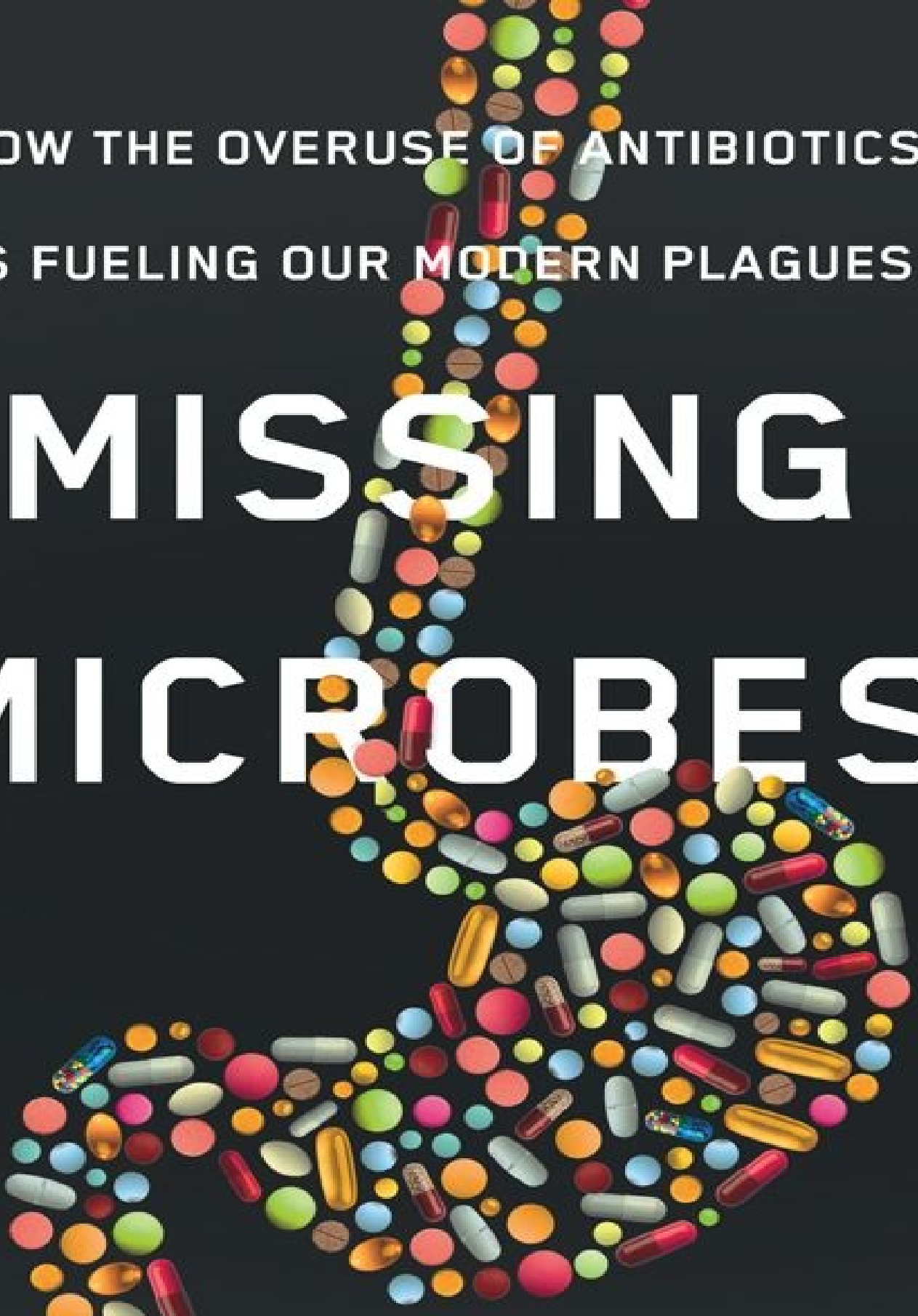
HOW THE OVERUSE OF ANTIBIOTICS IS FUELING OUR MODERN PLAGUES

MISSING MICROBES



MARTIN J. BLASER

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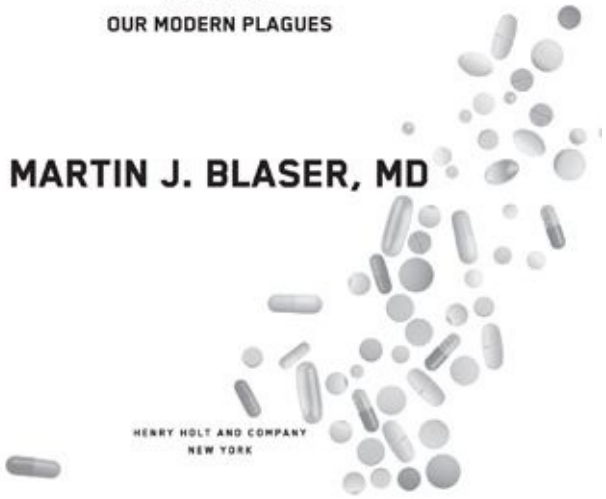
MARTIN J. BLASER, MD



HENRY HOLT AND COMPANY
NEW YORK

To my children, and to future children with a bright future

CONTENTS

Title Page
Copyright Notice
Dedication
Epigraph

1. Modern Plagues
2. Our Microbial Planet
3. The Human Microbiome
4. The Rise of Pathogens
5. The Wonder Drugs
6. The Overuse of Antibiotics
7. The Modern Farmer
8. Mother and Child
9. A Forgotten World
10. Heartburn
11. Trouble Breathing
12. Taller
13. … and Fatter

14. Modern Plagues Revisited
15. Antibiotic Winter
16. Solutions

Epilogue
Notes
Index
Acknowledgments
About the Author
Copyright

“We live in the Age of Bacteria (as it was in the beginning, is now, and ever shall be, until the world ends)…”

—STEPHEN JAY GOULD, Cambridge, MA, 1993

13.

… AND FATTER

Why do antibiotics make animals bigger and fatter? The goal in our study was to re-create the weight and size increases observed in farm animals in our lab and then tease out the principles for why they occur. It took a big team to address these questions, but several scientists played key roles: Ilseung Cho, a physician and a fellow in gastroenterology; Laurie Cox, a graduate student whose dissertation project revolved around the mouse models and who at age fourteen had started working with bacteria for her father's company, which made products for clinical bacteriology labs; and postcollege student Yael Nobel. Without such intelligent and dedicated trainees, I could not have tested any of my ideas. And there were many others who joined in the quest, from

high school and college students working during the summer to college students doing independent research and visiting scholars from around the world.

In 2007, after a number of attempts to get the model going, we began our first complete set of experiments on farm practices by adding four different subtherapeutic antibiotic treatments, which we called STAT, to the water bottles of mice. We only looked at females because they don't fight as much as males, making work easier for us. The early results were not promising; there was no weight difference between the STAT and control mice.

When Ilseung's research committee was told that the mice were not gaining weight, one of our experts asked, "What's happening to their body composition?" He was referring to the proportions of fat, muscle, and bone. We didn't know.

"Why don't you DEXA them and find out?" he asked.

DEXA them? The term refers to dual-energy X-ray absorptiometry, a test given to women to determine their bone mass and risk for osteoporosis. But DEXA also tells us how much fat is in the body and how much muscle.

This suggestion turned out to be critical. We discovered that all four groups of STAT mice had about 15 percent more fat than the controls, differences that could not be explained by chance alone.

We had our first evidence that antibiotics were

changing metabolism, affecting body composition. The STAT mice were making more fat and had about the same amount of lean muscle as the control. We also had an unexpected finding: at seven weeks of age, three weeks following the start of the antibiotics, the mice were putting on bone at an accelerated pace. More bone formation implies that they would become bigger, longer, and taller. But by ten weeks, all the mice had similar bone mass. The effect on bone showed up early only in those that were given antibiotics. In later experiments that I describe below, we also found bone effects, some of them lifelong. Again, this was not specific to a single antibiotic. If it was, one might think of it as a side effect of that one drug. But it was present across all the antibiotics tested. This work supports the idea that, in addition to better nutrition and clean water, antibiotics may be part of the explanation of why people are taller than ever.

We now had evidence that STAT changes early development but still did not understand how it happened. How did adding antibiotics to the water cause these developmental effects? What made the animals fatter and built up their bones earlier? We suspected that the drugs changed the composition of the intestinal resident microbes, so that is where we looked first by examining mouse poop. Fecal pellets represent the end product of everything that happens in the intestine and

could be collected every day from each mouse. The pellets gave us a standard material to compare in the same mouse over time, and across the mice that were exposed to different antibiotics or not, and varied diets.

We also studied material from an upper region of the colon called the cecum after sacrificing the animals. Cecal content was important to our study because it showed us which microbes were present and active in the body, not only after elimination in the feces. Because it had to be removed surgically, we could collect it only once, when the mice were killed. Most intestinal content of mice and humans, whether in the colon or in the poop, is undigested dietary fibers, water, and bacteria; the DNA present is nearly all bacterial. We performed what's called a universal bacterial 16S ribosomal RNA assay to learn more.

All bacteria share a gene that encodes 16S rRNA, which they need to make proteins. Although all bacteria have 16S rRNA genes, the exact DNA sequence substantially differs among bacterial species. The form in *E. coli* differs from that in *Staph*. So, by using the universal technique, followed by sequencing of the DNA products, we can take a census of "who is there." It is similar to taking a census in New York or Chicago and asking how many teachers, lawyers, police officers, and schoolchildren live there. In this case, we are asking how many clostridia, bacteroides, streptococci, and so on are

present, down to thousands of individual bacterial species. Based on the results of our census, we were able to address a number of important questions.

First, does the STAT treatment alter bacterial diversity? In other words, are the resident microbes of the antibiotic-treated mice as diverse as those of the control mice? Although both samples might be expected to have a lot of schoolteachers, students, and police officers, because they are common, will they have actuaries and piano tuners (rare professions) or had those dropped out?

We found that STAT, possibly because it is low dose, had no obvious effects on bacterial diversity. The same number of “professions” were present in the STAT-exposed and control specimens.

But what happens to the composition—the relative proportions of teachers, police officers, and so on—with STAT? We can take a census of who is there. For example, we would expect that the distribution of these professions in New York and Chicago would be closer in composition with each other than either would be with Delhi or Beijing. This is a model of what we find in the gut microbiome.

This is where things got interesting. STAT changed the composition of the intestinal microbial population, whether we examined the fecal pellets or the cecal contents. We expected that usual antibiotic exposures would change the mix, but we didn’t know whether very

low doses of STAT would do the same. We found that they did.

But did they change the functions of the bacteria? The answer is yes. Most of the food you eat is digested and absorbed in your small intestine. Residual food that reaches your large intestine is mostly indigestible. But here your bacteria come to the rescue. Recall that certain microbes in the colon digest this material and produce what are called short-chain fatty acids (SCFA), which are absorbed in the colon. These SFCA represent 5–15 percent of the calories you take in every day. If your microbes were more efficient at extracting calories from this “indigestible” food, then you would be better nourished. You might get fatter.

We measured SFCA levels in cecal contents and found that they were significantly greater in the STAT mice than in the controls. That meant that STAT mice were getting more calories early in life from their microbes, just as their tissues were developing.

We next zeroed in on the liver, the body’s main metabolic factory. It transforms the food absorbed in the intestinal tract, including the SCFA, into useful products, including proteins, energy sources such as sugars and starches, and energy-storage molecules such as fat. We compared the genes expressed in the livers of STAT mice to those of the control mice.

We were right on target. The liver in STAT mice up-

regulated the genes needed to make and transport more fat out to the periphery—the blubbery layers of fat animals. We knew that the STAT mice were putting down more fat and that it had to come from somewhere. The liver made sense. It is strategically interposed between the intestinal tract, where energy is acquired or generated, and the adipose tissue, where fat is stored.

*

Our next experiment, planned and carried out by Laurie, examined in more detail what happens when mice get antibiotics (we chose penicillin) very early in life. In Ilseung's experiment, animals got the drugs when they were weaned, about twenty-four days after birth. This is equivalent to at least twelve months for a human baby. Now Laurie gave antibiotics to the mothers during their pregnancy, so that their microbes, including those in the vagina, were altered from the get-go. The infant mice began life exposed to an altered microbiome, and we continued to give them antibiotics. As we predicted, the mice exposed at birth grew more than those exposed at day twenty-four. That became our standard way to conduct experiments.

Next, Laurie conducted an experiment that zeroed in on *when* the mice begin to get fat. Mice grow rapidly from the time they are born. Would they be putting on the

extra fat in their youth or did it take a while? The results of the experiment were clear. In males we found a difference from controls at sixteen weeks, and in females fat showed up at twenty weeks (middle age for a mouse). But in both sexes, once it was there, the increased fat persisted for their entire life span.

Subsequently Laurie looked at which species of bacteria were prevalent in these young mice. At four weeks, control animals were dominated by *Lactobacillus*, the bacteria originating in their mother's vagina. This was expected because the animals had just finished nursing, a time when, in both mice and humans, lactobacilli dominate.

But in the STAT group most of the lactobacilli were gone, replaced by other groups of bacteria. Since the changes in body composition were detected after sixteen weeks and the resident microbes were different at four weeks, we had a critical observation: changes in the microbiome preceded the changes in body composition.

Some elegant work done by my longtime friend and colleague Jeff Gordon at Washington University in St. Louis adds insight to our findings. Jeff has been a giant in the field of microbiome science, building on his years of research on how the gastrointestinal tract develops and functions. Jeff's group studied mice with a deletion in the gene responsible for the production of leptin, the "feed me" hormone that helps regulate appetite and helps the

brain decide whether to store or use energy. Leptin-deficient mice, called *ob/ob* mice, become markedly obese. Jeff and his colleagues asked whether the resident microbes of the *ob/ob* mice differ from those of their normal littermates. The answer was yes. Each type of mouse had different microbial populations in its guts.

Then Jeff asked if the microbes performed different metabolic roles. He transferred intestinal contents from the obese *ob/ob* and the normal mice into germ-free mice. These mice have thinner intestinal walls with fewer cells and do not gain as much weight. But when they are conventionalized and get microbes back, how well do they grow? Jeff's finding, which made news around the world, is that resident microbes taken from obese mice caused the recipient mice to put on fat at an accelerated rate compared to the mice that received microbes from the normal-weight mouse donor.

But here is something to consider: the mice in Jeff's experiments had a genetic defect that made them obese to begin with. That was the cause of the obesity; the change in the microbial populations was secondary. Although Jeff's team had beautifully characterized the consequences of obesity on the microbes and their functions, I did not think they were addressing the root cause of obesity. Moreover, germ-free mice, which provide an elegant system for testing specific hypotheses about immunity and metabolism, are completely

artificial. Yet although there are no natural germ-free mice or humans, we still can learn much about the fundamental principles of host-microbial interactions.

My own view was that antibiotic-induced perturbations in resident microbes early in life in relatively normal individuals might be the primary events that change host metabolism. (It would be about two years until we had more definite proof.)

Next, we asked what happens if we combine STAT with a high-fat diet. As we all know, our children's diets have gotten a lot richer in recent decades, whether from sugary drinks or from high-fat foods. They are taking in more calories on average than kids did one and two generations ago. We know that mice get fatter on a calorically rich diet, but would STAT increase or decrease the trend, or would it just be neutral?

Laurie called this experiment FatSTAT, and again the results were exciting. As we expected, mice on the high-fat diet got bigger than animals on normal chow. But adding antibiotics made a significant difference. We had mimicked the manner in which modern farmers are raising their livestock. Males on the combination (fat diet and antibiotic) were about 10 percent bigger still, having gained both muscle and fat. But the most striking differences were in the amount of body fat: with the combination, males had about 25 percent more, but females had an astounding 100 percent more. The

females on the high-fat diet gained about 5 grams of fat, whereas those on the fat diet and antibiotic gained 10 grams. They doubled their body fat. That's a lot, considering their total body weight was 20–30 grams.

Thus antibiotics had an effect, high-fat diet had an effect, but together they were more than additive; they were synergistic. For the female mice, the antibiotic exposure was the switch that converted more of those extra calories in the diet to fat, while the males grew more in terms of both muscle and fat. We do not yet know the reason for these sex differences. Nevertheless, the observations are consistent with the idea that the modern high-calorie diet alone is insufficient to explain the obesity epidemic and that antibiotics could be contributing.

We asked another simple question that Laurie's thesis committee suggested to us. Up to this point, we were keeping the animals on STAT for their entire lives. Would a few weeks of antibiotic treatment be enough for the weight gains to persist? This was a question important for our children's future. If the weight gain happens only after long-term treatment, then maybe this isn't relevant to our kids. Very few get lifelong antibiotics. But if short-term exposures cause the problem, this may be a way to explain our current epidemic. Most children are getting relatively short exposures of antibiotics for their ear and respiratory infections, especially early in life.

In March 2011, Laurie began the DuraSTAT experiments, so named because we were testing the durability of a brief antibiotic exposure to produce an effect. She divided the mice into four groups: no antibiotics, which was the control group; STAT for only four weeks and then stop; STAT for eight weeks and then stop; or STAT for the duration of the experiment. All of the mice were put on a high-fat diet at six weeks to bring out any differences. Laurie focused on females because of the results of our FatSTAT study.

Mice getting continuous antibiotics for the duration of the experiment gained weight compared to the controls, just as expected. But the effects of getting antibiotics for four weeks or eight weeks were the same as for twenty-eight weeks. The mice receiving the penicillin gained 10–15 percent more in total weight and 30–60 percent in fat compared to the antibiotic-free control mice. In other words, exposure to STAT early in life was sufficient for a lifelong effect; the development of the mice changed. Although the results in DuraSTAT were not identical to those of FatSTAT, neither were the experimental conditions. So the experiments are not directly comparable. The relevant comparisons are within each experiment. This is an important issue in science, where investigators have gone astray by comparing the effects in one experiment with those in a different one; conditions change in ways that often are not being

measured. But for us, the trends were exactly the same: early-life STAT permanently changed development in these mice.

Next we decided to study the microbiome itself. Laurie had been faithfully collecting the tiny fecal pellets, often once a day, from every mouse. She had thousands of little plastic test tubes in white boxes, one pellet per tube, one hundred tubes per box. It would take about eighteen thousand pellets to make a pound. They were worth more than their weight in gold because of the secrets they carried.

Laurie sequenced hundreds of specimens to determine their DNA compositions and to learn about the structure of their microbial communities (let’s say again assessing the ratio of teachers to police officers but now in much greater detail), including tax lawyers, taxi drivers, and taxidermists.

First she looked at samples from newly weaned three-week-old mice that had been given penicillin and compared them with samples from control mice that did not get the drug. Although the community structures of the two groups overlapped some, they were clearly different. This was exactly as we expected: antibiotics affect the structure of the microbial community in the intestinal tract.

Then we looked at pellets obtained eight weeks into the study. Now there were essentially three groups of

mice: the controls (no antibiotics), the mice still receiving antibiotics, and those who stopped antibiotics after four weeks and drank plain water for the next four weeks. As expected, the microbial community structure of controls and mice on continuous antibiotic were even more dissimilar than they were at three weeks. Antibiotics work. But the microbial community structures of the mice that had stopped taking antibiotics now looked just like those of the controls; they nearly overlapped. This means that the major effects of four weeks of antibiotics on the community structure were just transient. This was very clear. Yet, remember, these mice got just as fat as the others, which suggests that a brief exposure to antibiotics early in life, which causes an early perturbation of resident microbes, can lead to a lifelong effect. And the perturbation need not be permanent.

This is a key finding. I believe that it is the paradigm for what is happening to our children. Disturbing the microbes of mice during this critical early window is sufficient to change the course of their development. This was the experiment that proved to me that antibiotics have the potential to change development. And, of course, development is multidimensional: it is metabolic, as we were studying in the mice, but it is also immunologic and cognitive. As babies grow, while they are sleeping and dreaming, the context of their later development is being formed in partnership with their

ancient microbes. Even transient perturbations at that critical time can make a big difference.

But we are scientists, and we need to keep extending the story, learning the details, and finding the mechanisms. We need to answer the seemingly simple question: How does it work? What is so important about antibiotic exposure? Is it just its effects on the microbes, or is it because the penicillin had other effects on the body, directly interacting with the tissues of the mouse, irrespective of the effects on the microbes? As with many prior experiments, including those conducted by Jeff Gordon, we would attempt to answer the question by transferring microbes between mice.

Recall our earlier question: Was weight gain a direct effect of the antibiotics or was it a result of how the antibiotics affected resident microbes? We presumed it was the microbes, but presumption is not proof. To find out, we needed to transfer the STAT or the control microbes into a neutral situation and then observe whether there were differences in the recipients. Like Jeff, we chose to study the effects in germ-free mice.

We bought fifteen germ-free female mice, and in late August 2011 they arrived in three plastic bubbles, five to a bubble, newly weaned at three weeks old. The company told us that we could maintain them in the bubble for up to seventy-two hours, enough time to start our experiment. We called it TransSTAT because we were

transferring the STAT-affected microbiota to recipient mice.

Laurie chose six eighteen-week-old mice from her DuraSTAT experiment: three controls and three on continuous antibiotics. She collected cecal contents from each mouse and pooled them into two groups, one from controls, one from STAT mice. Calling on her extensive background in bacteriology, Laurie took special steps to preserve the viability of the microbes, some of which are so sensitive to oxygen that even a brief exposure to air kills them. Then she introduced the cecal matter into the stomach of each germ-free mouse. Seven received pooled cecal contents from controls, and eight received the cecal contents from STAT mice. To you and me the introduction of cecal contents into the stomach seems particularly unappetizing, but mice are coprophagic, meaning that they regularly eat their own feces as well as the feces of cohoused mice.

Now the mice were no longer germ-free. They had been “conventionalized” and could begin the next phase of their lives with their own residential microbes. We followed them for five weeks, obtaining frequent fecal samples and taking measurements, including DEXA scans, four times on each mouse. None of the mice received any antibiotics. All were raised identically, differing only in which microbes they received.

As expected, all of the mice gained weight since they

were still growing. However, the mice given the STAT microbes gained more weight and had more fat than the mice that were fed the control microbes. Nor were the effects small. The STAT recipients gained about 10 percent more weight and about 40 percent more fat than the control recipients did.

With this experiment, Laurie proved that the STAT-induced changes in development were transferrable by altered microbes alone.

*

STAT showed us what happens on the farm. But I am mostly interested in human children. When they get antibiotics, the dosage is rarely continuous. Rather, as discussed earlier, they receive short courses, usually five to ten days, depending on the problem (ear infection, bronchitis, sore throat) and on the doctor.

I wanted to see whether short pulses of antibiotics would affect weight gain and fat. Thus came the new model, which we called PAT, for pulsed antibiotic treatment. Instead of low doses, mice got the antibiotics just like children do, full therapeutic doses for just a few days in several pulses.

We chose amoxicillin and tylosin, which together represent more than 80 percent of all the antibiotics prescribed for American children. We then selected four

groups of mice: controls that did not get antibiotics, a group given amoxicillin in three pulses, a group given tylosin in three pulses, and—thinking there might be an additive effect—a mixed group that alternated tylosin, amoxicillin, tylosin for their three pulses.

To get the drugs into the baby mice as early as possible, Yael bred adult females and put antibiotics (or not, for the controls) into their water ten days after they gave birth. We guessed the drugs would be absorbed into the bloodstreams of the moms and get into their milk and thus affect the microbes in their pups—an assumption that proved to be correct.

The first PAT exposure occurred when the babies were ten to fourteen days old. At twenty-eight days old, after they were weaned and on their own, and again at thirty-seven days old, they got three-day pulses in their drinking water. On day forty-one, we switched all the mice to a high-fat diet, so we could enhance differences induced by antibiotics. All the mice we studied were females, because the breeding resulted in a more regular group than the males.

By day twenty-eight, all the PAT mice were growing significantly faster than the controls. We performed analyses on fat, bone, and muscle for the next 150 days of life, which took the mice well into middle age and early old age. PAT mice showed more muscle mass than the controls but not much difference in fat mass. Bone was a

different matter. The PAT mice that received amoxicillin showed increased bone area and mineral content for the duration of the experiment. Perhaps the effect was permanent because they received the drugs so early in life. And since amoxicillin is the most frequently prescribed drug in childhood, I can only wonder if that's the drug that most promotes the recent increases in human height.

Yael had collected more than three thousand fecal pellets from the mice and, for each specimen, knew which mouse it came from, on which day, and which treatment the animal got. With help from colleagues at Washington University in St. Louis we took a close look at their DNA. We wanted to know how our treatments had affected each animal's intestinal microbial diversity.

We found that moms had an average of 800 species in their fecal pellets. After one pulse, pups in the control group were like the moms. Those in the amoxicillin group had about 700 species. But mice in the tylosin and mixture group had only 200 species. In other words, the one course of antibiotics had caused the suppression or disappearance of about two-thirds of the usual bacteria in their feces. We saw a similar effect with amoxicillin, but it was much milder.

Now, after the three courses were over, we wondered whether the richness and biodiversity of the bacterial species would bounce back. It mostly did for amoxicillin,

which is a relatively mild drug. But in the mice that had received the tylosin, the diversity never went back to normal, even months after the last antibiotic dose. Tylosin had permanently suppressed or wiped out a proportion of the organisms passed on to them by their mother.

We also measured the so-called evenness of microbial diversity. If it's high, it means most species are found in roughly equivalent numbers. If low, only one or a few species dominate. In a human society, we could compare peacetime, when many different professions are well represented, and wartime, when there is a huge increase in the number of soldiers and corresponding decreases in all other professional groups. In war, the professional structure of society changes markedly. Tylosin treatment gave us the wartime equivalent with low evenness. PAT was causing permanent changes to the structure of the microbial community early in life, just as the mice were developing.

*

All told, our STAT and PAT experiments built a strong story that early-life antibiotics change the development of mice through their effects on resident microbes. But mice are not humans. We wanted to know if anyone was trying to link obesity with antibiotic use in young children.

Despite a profusion of published studies on childhood obesity—including investigations into birth weight, time spent watching TV, amount of exercise, exact details on every dietary nuance—as well as some big studies now under way, no one to our knowledge had ever asked about antibiotics.

Then my colleagues Drs. Leo Trasande and Jan Blustein heard about the Avon Longitudinal Study of Parents and Children (ALSPAC) study in Britain. Beginning in 1991 more than 14,500 pregnant women in the Avon Health District were recruited for a study. Their children, enrolled at birth, became a cohort that was studied for the next fifteen years. We were particularly interested in the kids who became overweight or obese.

Luckily for us, there was exactly one question on the questionnaire each parent filled out periodically. As part of a survey of the drugs to which the kids were exposed, they asked: “Did your child use any antibiotics in the preceding period?” It was asked when the kids were six, fifteen, and twenty-four months of age.

Nearly a third had received antibiotics in the first six months of life. By age two, three-quarters had been treated. Did the antibiotics make any difference? The calculations were complex, and it took an excellent statistician like Leo to make sense of them. Leo had to examine the effects of antibiotics while controlling for such factors as the baby’s initial weight and the mom’s

weight and whether the baby had been breast-or bottle-fed and for how long.

The upshot: children who received antibiotics in the first six months of life became fatter. We weren't surprised; the earlier in life, the stronger the effect on farm animals. Laurie had shown that early-life dosing was more important in the mice and that if we had to guess which time period would be most important for the development of human babies, it would be the first months of life.

So on the farm, in our mouse experiments, and in an epidemiologic study of human children, there was consistent evidence that early-life exposure to antibiotics could change development leading to larger size and more fat. We are testing more variations in mice, but the story continues to hang true, as we begin to fill in more and more details of the plot and the characters.

*

After the first epidemiologic study, Jan and Leo used ALSPAC to look at methods of birth delivery. Using parallel statistical analyses, they found that C-section births also were associated more with obesity. This was one of several studies concerning U.S., Canadian, Brazilian, and now English children published in 2011–13 that examined the same question. There were

differences in design and findings among all of the studies—for example, we found that nearly all of the effect occurred when the mom already was overweight. However, in each studied population C-section was associated with worse outcomes but the other (confounding) factors may also contribute to the risk. No one had ever made the connection between C-sections and childhood obesity. Maybe the informed consent form that a woman signs before she undergoes a C-section will say in the future: “One of the risks of this procedure is that your baby has an increased risk of becoming obese, and developing celiac disease, asthma, allergies…”

I list these other conditions here, because there have been convincing studies showing the relationship of C-section with these other modern plagues. Now that we know how medical interventions affect development and could lead to these diseases, perhaps we can find ways to prevent and treat them. But first, let’s examine them more closely.

EPILOGUE

Karl Benz, Henry Ford, and the other automotive inventors in the late nineteenth and early twentieth centuries made a monumental contribution to human life. They invented, perfected, and mass-produced the internal combustion engine, a machine that enables us to drive to work, to carry large loads, to go on holidays, to explore the world, and much more. Human existence has changed as a result: we are more interconnected, we can war at longer range, we can meet people of all different ethnicities and cultures.

We already know that the internal combustion engine also has spawned a host of new problems or worsened those we already had: air pollution, vehicular homicide, traffic jams. Perhaps Ford could have anticipated these; although they were unintended, they could have been

imagined. Cities with horse-drawn carriages had traffic tie-ups, and all of the excreta was not exactly pleasant. As such, many of the problems that followed the widespread introduction of the internal combustion engine were extensions of the known.

Yet imagine that about a hundred years ago someone told Henry Ford that every time a person turned the ignition in his or her car, the ice cap in Greenland would melt a little. It would have been unfathomable, and Ford likely would have dismissed it immediately. What if someone told *you* that same thought about thirty years ago? You probably also would have thought that it was ridiculous. How could those two occurrences be related? Yet we know how the unconnected become connected. This is one example of how our successful inventions are transforming our "macroecology," the status of our planet.

The story I have told concerns how we are changing our "microecology" with well-intended and indeed life-saving measures like antibiotics and Cesarian sections. That the resident microbes living in us are changing with disastrous results may seem as foreign as global warming would have been to Ford. But now, more than forty years after the "Earth" movement began, I believe that we are finally primed to contemplate and address these changes.

The ill effects in this story may be no less profound than those related to global warming and, in fact, may be

operating on a shorter time frame. I do not wish to ban antibiotics or Cesarian sections any more than anyone would suggest banning automobiles. I ask only that they be used more wisely and that antidotes to their worst side effects be developed. The truth is always obvious in retrospect. How could people really have thought that the sun revolves around Earth or that Earth is flat? Yet dogma are powerful and to their adherents infallible.

Once the question, Do antibiotics have a biological cost as well as clear benefits? is even posed, the horizon begins to shift. The answer is that, of course, our powerful antibiotics could affect our friendly bacteria. Of course, changing the mechanics of labor and delivery from the ancient ways to the modern in a third or half of our births today could have effects. Of course, purposely removing our natural microbial inhabitants is likely to have complex consequences.

The logic is inescapable. Our ancient microbes are there for a reason; that’s how we evolved. Everything that changes them has a potential cost to us. We have changed them plenty. The costs are already here, but we are only just beginning to recognize them. They will escalate.

The moment for substantive change is now. But change takes time, and reversal of the losses takes even longer. As with global warming, there is the risk that the status quo is “locked in.” Yet I am optimistic. The changes in human microecology have been going on for

only about a century and especially the past sixty to seventy years. This is the blink of an eye in the totality of human experience. Change that comes fast can depart just as rapidly.

We stand at the proverbial crossroads. We have medicines and practices that have served us well but have had unintended consequences. With powerful agents of any kind, there always are unintended consequences, so it should be no surprise. But the wake-up call is that we are not talking about uncommon events. The practices that endanger our children are at the core of modern health care.

We have made so much real progress in combating and eradicating terrible diseases. But now perhaps our efforts have peaked, and the fruits of discovery have left their seeds, indigestible and toxic. We must act, for the consequences are beginning to swallow us, and stronger storms lie ahead.

Yet many types of solutions are available. And with some of these, there may be synergies, combining the effects of two approaches, like curtailing both C-sections and antibiotic use, and eventually replacing disappeared organisms. For the future of our children and theirs, it is time for us to begin implementing them in earnest.

NOTES

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1. MODERN PLAGUES

have been getting healthier: In ancient times, one-third to one-half of children did not survive until the age of five. (See T. Volk and J. Atkinson, "Is child death the crucible of human evolution?" *Journal of Social, Evolutionary and Cultural Psychology* 2 [2008]: 247–60.) Childhood death rates remained high through the nineteenth century. Even by 1900, in some U.S. cities up to 30 percent of infants died without seeing their first birthday. (See R. A. Meckel, *Save the Babies: American Public Health Reform and the Prevention of Infant Mortality, 1850–1929* [Baltimore: Johns Hopkins University Press, 1990].) By the twentieth century, improved public health started to make a huge difference; infant mortality went from about 100/1,000 in 1915 to about 10 in 1995 (*Morbidity and Mortality Weekly Report* 48 [1999]: 849–58). Childhood mortality rates have continued to fall in the last half century (G. K. Singh and S. M. Yu, "U.S. childhood mortality, 1950 through 1993: trends and socioeconomic differentials," *American Journal of Public Health* 86 [1996]: 505–12).

worldwide obesity epidemic: Although increased body mass ultimately reflects more calories in than out, obesity is a complex issue. The question of whether all food calories are equal in terms of human metabolism is controversial. Issues

such as physical and psychological stress and lack of sleep may affect *(increasing)* food intake. Lack of exercise may play a role in weight gain disproportionate to its direct effect on calorie expenditure. Maternal smoking, prenatal environment, hormone disruptors, and salted-food addiction all have been postulated as causative, and even chemical toxins have been considered to play a role. (P. F. Baillie-Hamilton, "Chemical toxins: a hypothesis to explain the global obesity epidemic," *Journal of Alternative and Complementary Medicine* 8 [2002]: 185–92.) **has risen 550 percent since 1950:** In developed countries, juvenile (Type 1) diabetes has been steadily rising. (V. Harjutsalo et al., "Time trends in the incidence of type 1 diabetes in Finnish children: a cohort study," *Lancet* 371 [2008]: 1777–82.) Although, after more than fifty years of continued growth and a recent period of accelerated growth, the incidence appears to be leveling off, possibly because of public-health activities. (V. Harjutsalo et al., "Incidence of type 1 diabetes in Finland," *Journal of the American Medical Association*, 310 [2013]: 427–28.) Worldwide, the annual increase in Type 1 diabetes in recent years has been about 3 percent. (P. Onkamo et al., "Worldwide increase in incidence of Type I diabetes—the analysis of the data on published incidence trends," *Diabetologia* 42 [1999]: 1395–403.) **resemble those of adults:** T. Yatsunenko et al., "Human gut microbiome viewed across age and geography," *Nature* 486 (2012): 222–27. In this study, after comparing the gut microbiota from people in the United States, Malawi, and Venezuela (Amerindians), researchers found that the compositions in infants were markedly different from those in adults. But as children matured, their microbiomes became more and more adultlike. Importantly, the age at which this happens is three. The transition from no microbiota to an adultlike microbiota is all accomplished during the earliest stages of life, just as many functions in the host are developing.

the "disappearing microbiota": The disappearing-microbiota hypothesis evolved over a number of years. A few of my key papers that develop the theme include: "An endangered species in the stomach," *Scientific American* 292 (February 2005): 38–45; "Who are we? Indigenous microbes and the ecology of human disease," *EMBO Reports* 7 (2006): 956–60; with my very distinguished colleague Stanley Falkow, "What are the consequences of the disappearing microbiota?" *Nature Reviews Microbiology* 7 (2009): 887–94; "Stop killing our beneficial bacteria," *Nature* 476 (2011): 393–94.

"cloak of invisibility": The discovery of the stealth mechanisms of

Campylobacter fetus involved a progressive series of experiments conducted over nearly twenty years. A few of the key papers include: M. J. Blaser et al., "Susceptibility of *Campylobacter* isolates to the bactericidal activity in human serum," *Journal of Infectious Diseases* 151 (1985): 227–35; M. J. Blaser et al., "Pathogenesis of *Campylobacter fetus* infections. Failure to bind C3b explains serum and phagocytosis resistance," *Journal of Clinical Investigation* 81 (1988): 1434–44; J. Dworkin and M. J. Blaser, "Generation of *Campylobacter fetus* S-layer protein diversity utilizes a single promoter on an invertible DNA segment," *Molecular Microbiology* 19 (1996): 1241–53; J. Dworkin and M. J. Blaser, "Nested DNA inversion as a paradigm of programmed gene rearrangement," *Proceedings of the National Academy of Sciences* 94 (1997): 985–90; Z. C. Tu et al., "Structure and genotypic plasticity of the *Campylobacter fetus sap* locus," *Molecular Microbiology* 48 (2003): 685–98.

and house cats (*Felis catus*): Unfortunately, taxonomy is often complicated because our house cats also have been classified as *Felis silvestris*, within the species of wildcats, or sometimes called *F. silvestris f. catus*. Still, a cat by any other name would meow.

natural defenses against it: Based on our studies of variation in campylobacters and host responses to them, we began to study the same for the gastric campylobacter-like organism (or GCLO), which for a time was called *Campylobacter pyloridis*, then *Campylobacter pylori*, before eventually its current name, *Helicobacter pylori*, was agreed upon. Our first papers about this were: G. I. Pérez-Pérez, and M. J. Blaser, "Conservation and diversity of *Campylobacter pyloridis* major antigens," *Infection and Immunity* 55 (1987): 1256–63; and G. I. Pérez-Pérez, B. M. Dworkin, J. E. Chodos, and M. J. Blaser, "*Campylobacter pylori* antibodies in humans," *Annals of Internal Medicine* 109 (1988): 11–17. From these studies we developed a blood test (which is the basis for most of the blood tests used today in the United States) to diagnose whether or not a person has *H. pylori* in his or her stomach.

"the only good *H. pylori* is a dead *H. pylori*": In response to my paper in the *Lancet* (M. J. Blaser, "Not all *Helicobacter pylori* strains are created equal: should all be eliminated?" *Lancet* 349 [1997]: 1020–22), David Graham wrote to the editor: "The only good *Helicobacter pylori* is a dead *Helicobacter pylori*" (*Lancet* 350 [1997]: 70–71). This became the signature concept for the present era.

our normal gut flora: *Flora* is the old name for the countless organisms that live in humans. We used to call them our normal flora. But bacteria are not plants, and the organisms that live in and on us are both small and diverse. We now call these organisms our *microbiota*. And all of the relationships between the microbiota and ourself, and with each other, are collectively called the *microbiome*.

2. OUR MICROBIAL PLANET

"his middle finger erases human history": J. McPhee, *Basin and Range*, book 1 in *Annals of the Former World* (New York: Farrar, Straus & Giroux, 1998).

a few exceptions that reinforce the rule: H. N. Schulz et al., "Dense populations of a giant sulfur bacterium in Namibian shelf sediments," *Science* 284 (1999): 493–95. But such large microbes are the anomalies in a world dominated by microscopic forms.

the distance between corn and us: N. Pace, "A molecular view of microbial diversity and the biosphere," *Science* 276 (1997): 734–40. To Carl Woese, Norman Pace, and many others, bacteria were at the very origins of all life on Earth.

240 billion African elephants: W. B. Whitman et al., "Prokaryotes: The unseen majority," *Proceedings of the National Academy of Sciences* 95 (1998): 6578–83; J. S. Lipp et al., "Significant contribution of Archaea to extant biomass in marine subsurface sediments," *Nature* 454 (2008): 991–94; and M. L. Sogin et al., "Microbial diversity in the deep sea and the underexplored 'rare biosphere,'" *Proceedings of the National Academy of Sciences* 103 (2006): 12115–20.

selection in action: Plastic-eating bacteria. T. Suyama et al., "Phylogenetic affiliation of soil bacteria that degrade aliphatic polyesters available commercially as biodegradable plastics," *Applied and Environmental Microbiology* 64 (1998): 5008–11; E. R. Zettler et al., "Life in the 'plastisphere': microbial communities on plastic marine debris," *Environmental Science and Technology* 47 (2013): 7137–46.

water, and bacteria—loads of them: T. O. Stevens and J. P. McKinley, "Lithoautotrophic microbial ecosystems in deep basalt aquifers," *Science* 270

(1995): 450–54.

the common intestinal bacterium *E. coli*: *E. coli's* formal name is *Escherichia coli*, honoring Theodor Escherich, a German doctor who discovered it in 1885 in the feces of healthy people, and called it *Bacterium coli commune.* In the early twentieth century, the name was changed to *Escherichia coli.* Although the best-known bacteria in the human gastrointestinal tract, it usually represents less than one-thousandth of all the bacterial cells present. While most *E. coli* strains are harmless, there are distinct strains that can cause several different types of disease. Because of the ease of growing *E. coli* in culture, it has become a model organism to study the biology, biochemistry, and genetics of cellular life. Many of the five thousand genes in *E. coli* cells have analogues in human cells.

"and ever shall be, until the world ends": In 1993 S. J. Gould wrote a review that appeared in *Nature* about E. O. Wilson's then new book *The Diversity of Life*, in which he indicates that Wilson already knows that rather than an individual age of reptiles or of mammals, these are but parts of the eternal age of bacteria, as he so states. (S. J. Gould, "Prophet for the Earth: Review of E. O. Wilson's 'The diversity of life'," *Nature* 361 [1993]: 311–12.)

3. THE HUMAN MICROBIOME

They are symbionts: Symbiosis, defined in the nineteenth century, is the close relationship of two (or more) species living together, sometimes for most or all of their lifetimes. Although it may mean living together harmfully, neutrally, or helpfully, it also can be used to describe just the mutually helpful relationships. A species party to such a relationship is a symbiont.

Aphids, small insects that live on plants: N. Moran, "The evolution of aphid life cycles," *Annual Review of Entomology* 37 (1992): 321–48.

more apelike than cowlike: H. Ochman et al., "Evolutionary relationships of wild hominids recapitulated by gut microbial communities," *PLOS Biology* 8 (2010): e1000546.

Of fifty known phyla: A phylum is a term in biology referring to the taxonomic classification between kingdom and class. The kingdom Anamalia, encompassing all animals, has about thirty-five phyla, ranging from Arthropoda

(insects) to Chordata (having a spinal cord, like humans).

in your mother's womb, you had no bacteria: This has been the long-held belief, but evidence is beginning to emerge that even in the womb in many animals microbes are normally present (L. J. Funkhouser and S. Bordenstein, "Mom knows best: the universality of maternal microbial transmission," *PLOS Biology* 11 [2013]: e1001631). However, this is still an area of controversy. We will probably know for sure, one way or the other, in humans in a couple of years.

over the first three years of life: In a study of the gut microbiota of healthy people in three places—the United States, Malawi, and Venezuela (Amerindians)—Yatsunenko and her colleagues, including my wife, Gloria, catalogued which microbes were present across people of all ages. In early life, there were great similarities between the three different ethnic groups, but as they got older they diverged. Perhaps most important, the composition of the microbiota in infants is very different from that of adults, but gradually it becomes more and more adultlike, reaching adult levels by the age of three! (T. Yatsunenko et al., "Human gut microbiome viewed across age and geography," *Nature* 486 [2012]: 222–27.) Initially I was surprised, but the more I thought about it, the more sense it made—the microbiome develops in parallel with the development of the child. This was consistent with my hypotheses about the importance of the early-life microbiota.

are home to different species: We did the first survey of the skin using molecular methods beginning in 2004 and showed the incredible diversity but also the symmetry between left and right. (Z. Gao et al., "Molecular analysis of human forearm superficial skin bacterial biota," *Proceedings of the National Academy of Sciences* 104 [2007]: 2927–32.) Then, using more powerful methods, other investigators confirmed and extended the observations, showing more subtle differences between left and right hands, and how our computer keyboards carry the microbial signatures of our fingertips—that is, we can tell your keyboard from mine (N. Fierer et al., "Forensic identification using skin bacterial communities," *Proceedings of the National Academy of Sciences* 107 [2010]: 6477–81). They also showed that each of the three major types of skin—dry, moist, and oily—has its own major populations (E. A. Grice et al., "Topical and temporal diversity of the human skin microbiome," *Science* 324 [2009]: 1190–92), and that a single group of fungi dominate in most of our skin, except

for the bottom of our feet (K. Findley et al., “Topographic diversity of fungal and bacterial communities in human skin,” *Nature* 498 [2013]: 367–70).

250 healthy young adults: The large Human Microbiome Project sponsored by the National Institutes of Health made incredible progress in laying out the fundamentals of our microbial composition. In the important study of healthy young adults in the United States (actually in Houston and St. Louis), the outlines of the human microbiome were shown. (C. Huttenhower et al., “Structure, function and diversity of the healthy human microbiome,” *Nature* 486 [2012]: 207–14.) In this paper, there were nearly as many authors (me included) as there were subjects, but it was a very complex “big science” national effort that paid large dividends—and that will keep paying as more and more scientists use the trove of information accumulated, from sampling at sixteen sites in men and women, plus three vaginal sites in the women. From that study, for example, we know much more about the populations in the mouth: how the top of the tongue, hard palate, and cheek are more similar to one another than to the gingival crevices.

they don’t like oxygen: The microbial composition of the gingival crevice is vast: its density is similar to that in the colon, and the variety of bacteria is enormous (I. Kroes et al., “Bacterial diversity within the human subgingival crevice,” *Proceedings of the National Academy of Sciences* 96 [1999]: 14547–52; and ibid.). That interface between tooth and gum is where periodontal disease occurs, and the hope is that by better understanding the microbial populations and their dynamics, we will be better able to prevent or treat this major cause of tooth loss.

who is attractive to mosquitoes: N. O. Verhulst et al., “Composition of human skin microbiota affects attractiveness to malaria mosquitoes,” *PLOS ONE* 6 (2011): e28991.

dozens of species living there: Z. Pei et al., “Bacterial biota in the human distal esophagus,” *Proceedings of the National Academy of Sciences* 101 (2004): 4250–55. Until we published our paper, no one thought that the esophagus had any residential bacteria, only transients traveling from the mouth and throat down.

colonic bacteria and in their functions: Just as we can construct family trees of plants and animals, using new computational tools, we can do the same for the

bacterial populations living in different ecological niches. We can compare the composition of microbial populations living in freshwater ponds to those in the oceans. (Not surprisingly, they are quite different.) When such tools are applied to the compositions of the colonic microbes in, for example, mice and humans, we can see enormous parallels (R. E. Ley et al., "Worlds within worlds: evolution of the vertebrate gut microbiota," *Nature Reviews Microbiology* 6 [2008]: 776–88). At higher taxonomic levels, starting at the phylum, we are nearly identical, but as we descend the phylogenic ladder, the differences become greater until, at the species level, mouse and human are highly distinct. In a way, these microbial similarities and differences capture our evolution from a common ancestor to distinct species—*Mus musculus* and *Homo sapiens*—as well as our own genetic inheritance. Yes, even our cohabiting microbes inform us that "ontogeny recapitulates phylogeny," a concept in evolutionary biology I learned as a high school student, long before I could even guess what evolution was all about.

the activities of your microbes: W. R. Wikoff et al., "Metabolomics analysis reveals large effects of gut microflora on mammalian blood metabolites," *Proceedings of the National Academy of Sciences* 106 (2009): 3698–703. Investigators compared germ-free mice (born in bubbles and without any bacteria at all) and more conventionally raised mice. They used very sensitive chemical sampling and detection methods to examine the contents in the bloodstreams of the two groups of mice. Of nearly 4,200 chemical constituents, only 52, slightly more than 1 percent, were seen in the blood of the germ-free animals; the more than 4,000 others were ultimately derived from bacterial metabolism. These studies provided evidence that most of the chemical constituents in the blood of mice (and thus by extension in us) are ultimately derived from having microbiota and from their interaction with our cells.

first chemical processing and then absorption: H. J. Haiser et al., "Predicting and manipulating cardiac drug inactivation by the human gut bacterium *Eggerthella lenta*," *Science* 341 (2013): 295–98.

at least for a short period: R. Avallone et al., "Endogenous benzodiazepine-like compounds and diazepam binding inhibitor in serum of patients with liver cirrhosis with and without overt encephalopathy," *Gut* 42 (1998): 861–67.

which is low in protein: The sweet potato is about 2 percent protein, so an adult would have to eat about five pounds of sweet potato a day to ingest enough

protein.

essentially lacks *Lactobacillus:* J. Ravel et al., "Vaginal microbiome of reproductive-age women," *Proceedings of the National Academy of Sciences* 108, suppl. 1 (2011): 4680–87.

gut microbiome is relatively stable: J. Faith et al., "The long-term stability of the human gut microbiota," *Science* 341 (2013): DOI: 10.1126/science.1237439. By studying the same people over time, often years, Jeff Gordon's lab showed that although there is turnover in the organisms that can be detected, there also is considerable stability. In their study, about 70 percent of the organisms present in adults on sampling were estimated to be present one year later.

the changes in microbial populations were more significant: Dr. Nanette Steinle from the University of Maryland presented the data on the dry bean/lentil study at the American Society for Nutrition's poster session on April 23, 2013. In other work, immediate diet effects on the microbiome were seen, but with long-term stability of overall composition. (See G. Wu et al., "Linking long-term dietary patterns with gut microbial enterotypes," *Science* 334 [2011]: 105–8.) **only as long as the person was consuming the special diet**: L. A. David et al., "Diet rapidly and reproducibly alters the human gut microbiome," *Nature* (2013): DOI 10.1038/nature12820.

millions of unique genes: Just as the human microbiome has been the focus of "big science" in the United States, another large group coalesced in Europe, the MetaHit consortium. They have done important work that is both unique and complementary to the findings of the HMP. J. Qin *et al.* ("A human gut microbial gene catalogue established by metagenomic sequencing," *Nature* 464 [2010]: 59–65) showed the huge range in composition from person to person. M. Arumugan *et al.* ("Enterotypes of the human gut microbiome," *Nature* 473 [2011]: 174–80) postulated that humans could be divided into three major types based on the composition of their gut microbiome, perhaps analogous to human blood types. Whether the typing scheme will stand up over time and whether the types are relatively stable in an individual host remain to be determined.

bacterial genes in subjects' guts varied dramatically: In a recent paper by the MetaHit group (E. Le Chatelier et al., "Richness of human gut microbiome correlates with metabolic markers," *Nature* [2013]: 500, 541–46), 292 subjects were studied in terms of their gut microbial gene counts and their metabolic

status. The results clearly show the gene count for the two groups: high for about three-quarters of the subjects and low for the remaining quarter. On average the people in these two groups differ significantly in their metabolic status. Those in the low-gene-count group were much more likely to have the metabolic syndrome, a constellation of findings associated with obesity, diabetes, hardening of the arteries, and high blood pressure. One question that could not be resolved by the study is which came first, low gut microbial gene count or the metabolic syndrome. But a companion paper showed that dietary interventions that improve metabolic status raise the gene count (A. Cotillard et al., "Dietary intervention impact on gut microbial gene richness," *Nature* 500 [2013]: 585–88).

staggering ten million–fold: Qin et al., "A human gut microbial gene," showed this.

never before encountered: See I. Cho and M. J. Blaser, "The human microbiome: at the interface of health and disease," *Nature Reviews Genetics* 13 (2012): 260–70, where we more fully discuss the concept of contingency organisms.

cows and their rumen bacteria: The rumen is the specialized, first stomach in ruminants like cows and sheep. It is a specialized compartment in which the microbes that are present ferment the ingested feed, allowing its energy to be digested by the host. The rumen is also a prime example of symbiosis, and the resident microbes include bacteria, fungi, protozoa, and viruses.

if you played fair and square: See M. J. Blaser and D. Kirschner, "The equilibria that allow bacterial persistence in human hosts," *Nature* 449 (2007): 843–49, for a fuller exposition of these ideas about equilibrium relationships between our microbes and us.

4. THE RISE OF PATHOGENS

causing a form of encephalitis: Encephalitis means inflammation of the brain. It is usually an acute infection caused by a virus or a bacterium, but may be due to other organisms, or may be noninfectious.

eat their prey from within: D. Quammen, *Spillover: Animal Infections and the*

Next Human Pandemic (New York: W. W. Norton & Company, 2012).

and killed fifty: The outbreak came out of nowhere, and uncounted thousands were exposed to the contaminated sprouts. A medical description of the outbreak was published in U. Buchholz et al., "German outbreak of *Escherichia coli* O104:H4 associated with sprouts," *New England Journal of Medicine* 365 (2011): 1763–70; and a description of the characteristics of the strain in C. Frank et al., "Epidemic profile of Shiga-toxin-producing *Escherichia coli* O104:H4 outbreak in Germany," *New England Journal of Medicine* 365 (2011): 1771–80; and how it all happened in M. J. Blaser, "Deconstructing a lethal foodborne epidemic," *New England Journal of Medicine* 365 (2011): 1835–36.

epidemic diseases began to take off: W. McNeill, *Plagues and Peoples* (New York: Anchor, 1977).

might infect from one-third to one-half of those exposed for the first time: A nineteenth-century accounting of what happened when measles came to an isolated island was by Peter Panum in his classic "Observations Made During the Epidemic of Measles on the Faroe Islands in the Year 1846" (*Bibliothek for Laeger*, Copenhagen, 3R., 1 [1847]: 270–344). There also have been more recent observations, e.g., when a boat landed in Greenland in the 1940s with a crew member who had measles.

18 deaths every hour: World Health Organization data on measles and deaths, http://www.who.int/mediacentre/factsheets/fs286/en/. Measles, the relatively mild and nearly universal childhood disease in the developed world until an effective vaccine was introduced in the 1990s, shows a very different face in developing countries. There, in the setting of malnourishment, immunodeficiency, and concurrent infections, measles is a killer. Each year, more than one hundred thousand children die as a result of measles. It is a calamity and one that vaccine can prevent. But the problems in deploying the vaccine to all in need have been political, logistic, and economic.

human population of 500,000 people: Decades before it entered the mainstream, Francis Black was one of the first to think about island biogeography in terms of the spread of infectious diseases in humans. (See F. L. Black, "Measles endemicity in insular populations: critical community size and its evolutionary implication," *Journal of Theoretical Biology* 11 [1966]: 207–11.) **the measles virus quickly spread from person to person:** Panum,

“Observations Made During the Epidemic of Measles.”

grain bins and trash heaps: M. J. Blaser, “Passover and plague,” *Perspectives in Biology and Medicine* 41 (1998): 243–56.

broke out in Kinshasa, Zaire: Not only in the fourteenth century but also in this one, plague still visits cities when the conditions are ripe for it. In Africa and India there has been urban plague in recent years. See, for example, G. Butler et al., “Urban plague in Zaire,” *Lancet* 343 (1994): 536; and details of a continued endemic focus: P. Boisier et al., “Epidemiologic features of four successive annual outbreaks of bubonic plague in Mahajanga, Madagascar,” *Emerging Infectious Diseases* 8 (2002): 311–16.

Twenty percent of children did not survive: Several different techniques were used to measure mortality. Extensive work was done by Samuel H. Preston and Michael R. Haines on estimating childhood mortality. See the chapter “New Estimates of Child Mortality During the Late-Nineteenth Century” in their book *Fatal Years: Child Mortality in Late-Nineteenth Century America* (Princeton: Princeton University Press, 1991), 49–87.

5. THE WONDER DRUGS

difficult-to-treat malignancy: In several studies, working with colleagues in Hawaii, at the Mayo Clinic, and in Japan, we had shown that people carrying that bacterium in their stomachs were more likely to have or to later develop stomach cancer (A. Nomura et al., “*Helicobacter pylori* infection and gastric carcinoma among Japanese Americans in Hawaii,” *New England Journal of Medicine* 325 [1991]: 1132–36; N. Talley et al., “Gastric adenocarcinoma and *Helicobacter pylori* infection,” *Journal of the National Cancer Institute* 83 [1991]: 1734–39; M. J. Blaser et al., “*Helicobacter pylori* infection in Japanese patients with adenocarcinoma of the stomach,” *International Journal of Cancer* 55 [1993]: 799–802). Other studies, conducted in England by David Forman and in California by Julie Parsonnet, showed very similar results. In a couple of years, we changed what people knew about the cause of stomach cancer, then and now the number-two cause of cancer death in the world (after lung cancer). We now know that more than 80 percent of all stomach cancer cases can be attributed to *H. pylori* (see chapter 9).

from white blood cells and from saliva: Fleming discovered lysozyme, a constituent of innate immunity, in saliva. It is an enzyme that by breaking the chemical bonds that hold the cell walls of bacteria together effectively dissolves (lyses) bacterial cells. It was a major discovery (in retrospect) of one of the arms of our innate (inborn) immunity. We have evolved a variety of molecules, like lysozyme, that have antagonistic activity against whole classes of bacteria. They reduce contamination on our mucosal surfaces, our coastlines, and also help clear tissues of invading bacteria. But most important, Fleming's discovery of lysozyme prepared him to recognize the lytic activities possessed by the accidental mold that landed on his plates a few years later. (A. Fleming, "On a remarkable bacteriolytic element found in tissues and secretions," *Proceedings of the Royal Society, Series B* 93 [1922]: 306–17.) **They had been inoculated with staph:** "Staph" is a nickname used by health professionals to refer to *Staphylococcus*. Usually this is in reference to *Staph aureus*, a major pathogen, rather than "*Staph epi*" (*S. epidermidis*), an important colonizer of the skin, with low virulence.u**sed molds to treat infected wounds:** Gloria's grandmother, who lived in rural Spain in the early twentieth century, would use moldy bread to help infections heal; it was common knowledge among the peasants, but never explored for how it worked.

After publishing his results: A. Fleming, "On the antibacterial action of cultures of a penicillium, with special reference to their use in isolation of B. influenzae," *British Journal of Experimental Pathology* 10 (1929): 226–36.

the first sulfonamide: Sulfonamidochrysoidine, the red dye called Prontosil, was shown by Domagk in 1932 to protect mice from *Strep*. It had actually been discovered more than twenty years earlier, but its medical use was not tested then. In 1935, a French group found that Prontosil was a pro-drug, metabolized to sulfanilamide, the active agent.

but not good enough: Co-trimoxazole, the drug that was successfully used to treat my case of paratyphoid fever, was actually a derivative of those original sulfa drugs. But they worked much better in combination than did the early forms of the drugs in the 1930s and '40s.

a way of growing the penicillium molds: When I later visited the Pfizer plant and research center in Groton, Connecticut, in the mid-1990s, the air was redolent with the odor of molasses. Why that sweet characteristic smell? Oceangoing ships from the West Indies would come up the Thames River, and

each would dock with its hold filled with molasses, which was used as the main source of food for giant vats of the *Penicillium* mold that itself manufactured the life-saving penicillin.

made by one living form to fight against another: Penicillin, the first antibiotic, was produced by a mold as an antibacterial substance. The sulfa drugs were made by chemical synthesis in a factory. They are not technically antibiotics, because they are synthetic, but we use the term antibiotics generically to include both true antibiotics and chemically synthesized agents (same for fluoroquinolones, like cipro).

6. THE OVERUSE OF ANTIBIOTICS

5.5 million stoves: The statistics about our prosperity and pent-up demand in 1945–1949 come from the Public Broadcasting System's show *The American Experience* and the episode "The Rise of American Consumerism."

derives from these ultracontagious human viruses: See T. M. Wassenaar and M. J. Blaser, "Contagion on the Internet," *Emerging Infectious Diseases* 8 (2002): 335–36, for a discussion of the parallels between infectious diseases and so-called computer viruses, the malware that is transmissible from person to person (or rather from computer to computer).

after a couple of weeks: For a natural history of cough in URIs, see: S. F. Dowell et al., "Appropriate use of antibiotics for URIs in children, Part II: Cough, pharyngitis and the common cold," *American Family Physician* 58 (1998): 1335–42.

to ward off rheumatic fever: The purpose of antibiotics for treatment of children with strep throat or what appears to be strep throat is complex. In S. T. Shulman et al., ("Clinical practice guideline for the diagnosis and management of Group A streptococcal pharyngitis: 2012. Update by the Infectious Diseases Society of America," *Clinical Infectious Diseases* 55 [2012]: e86–102), the IDSA guidelines committee makes important recommendations that I paraphrase: they note that testing for Group A strep (GAS) usually is not recommended for children or adults who have an acute sore throat with clinical features that strongly suggest a viral cause (e.g., cough, runny nose, hoarseness, and mouth ulcers). Diagnostic studies for GAS are usually not indicated for

children under three years old because acute rheumatic fever is rare in children under three, and the incidence of strep throat is uncommon in this age group. Laboratory confirmation is essential in making a precise diagnosis because physicians often greatly overestimate the probability that GAS is the cause of sore throat. A test that is negative for GAS provides reassurance that the patient's sore throat likely has a viral cause. While treatment early in the course leads to a more rapid clinical cure in patients with acute GAS pharyngitis and decreases transmission of GAS to other children, the predominant rationale for treatment of this self-limited illness is the prevention of acute rheumatic fever and other complications. The committee states that efforts to identify GAS carriers are not ordinarily justified, nor do carriers generally require antimicrobial treatment because they are unlikely to spread strep throat to their close contacts and are at little or no risk for developing acute rheumatic fever.

follow the safer course: Recommendations by the American Academy of Pediatrics (AAP) regarding antibiotic use have been in place for a long time. (See S. F. Dowell et al., "Principles of judicious use of antimicrobial agents for pediatric upper respiratory tract infections," *Pediatrics* 101, suppl. 1 [1998]: 163–65.) An important update was issued last year: A. S. Lieberthal et al., "The diagnosis and management of acute otitis media," *Pediatrics* 131 (2013): e964–99.

patients with pneumonia: W. S. Tillett et al., "The treatment of lobar pneumonia with penicillin," *Journal of Clinical Investigation* 4 (1945): 589–94.

antibiotics to people in the United States: L. Hicks et al., "US outpatient antibiotic prescribing, 2010," *New England Journal of Medicine* 368 (2013): 1461–62.

in the United States and other developed countries: For antibiotic use in other developed countries, for example, see M. Sharland, "The use of antibacterials in children," *Journal of Antimicrobial Chemotherapy* 60, suppl. 1 (2007): i15–i26.

MRSA stands for methicillin-resistant *Staphylococcus aureus*: MRSA infections were noted in the 1960s, almost immediately after antibiotics like methicillin were used to treat people with *Staph aureus* infections. But such drugs were mostly used in hospitalized patients, and MRSA strains were largely confined to the hospital. But in recent years, MRSA has been spreading in the community. These days, among serious staph infections in people coming to

emergency rooms for treatment, about 80 percent are due to MRSA. (G. J. Moran et al., "Methicillin-resistant *S. aureus* infections among patients in the emergency department," *New England Journal of Medicine* 355 [2006]: 666–74.) This is a dramatic change from the past. Resistance in *Staph* is spreading so greatly that the natural divisions between hospital and community have been blurred. But in fact the dominant MRSA strains are different. There are two largely separate populations of MRSAs, each adapted to its own ecological niche but each driven and selected by the enormous antibiotic pressures in both hospital and community.

"a tiny little thing that I can not see": Brandon Noble, IDSA website: http://www.idsociety.org/Brandon_Noble/.

NCAA Division III championships: Ricky Lannetti, MRSA awareness website: http://www.mrsaawareness.com/mrsaawareness/Home.html.

7. THE MODERN FARMER

an endless game of chemical warfare: See extended discussion of the arms races in chapter 2.

which have similar core structures: See V. D'Costa et al., "Antibiotic resistance is ancient," *Nature* 477 (2011): 457–61; and K. Bhullar et al., "Antibiotic resistance is prevalent in an isolated cave microbiome," *PLOS ONE* 7 (2012): e34953.

resistance from our activities: By studying large fish, which feed on smaller fish and are living on top of the food chain, scientists can most easily assess antibiotic contamination in the ocean. A recent survey found resistance in all six sites sampled and in all eight fish species studied. (See J. K. Blackburn et al., "Evidence of antibiotic resistance in free-swimming, top-level marine predatory fishes," *Journal of Zoo and Wildlife Medicine* 41 [2010]: 7–15.) **animals fed a drug-free diet:** The idea that antibiotics could be useful as growth promoters was first developed in the 1940s, shortly after their first deployment to treat infections in both humans and animals: P. R. Moore and colleagues ("Use of sulfasuxidine, streptothricin, and streptomycin in nutritional studies with the chick," *Journal of Biological Chemistry* 165 [1946]: 437–41) are generally credited for this observation. W. J. Visek ("The mode of growth promotion by

antibiotics," *Journal of Animal Sciences* 46 [1978]: 1447–69) wrote an outstanding review of the accumulated knowledge about thirty-five years ago; in the light of today's knowledge, the observations seem very accurate. Also see P. Butaye et al., "Antimicrobial growth promoters used in animal feed: effects of less well-known antibiotics on gram-positive bacteria," *Clinical Microbiology Reviews* 16 (2003): 175–88; E. Ozawa, "Studies on growth promotion by antibiotics," *Journal of Antibiotics* 8 (1955): 205–14.

a particularly interesting study from 1963: M. E. Coates et al., "A comparison of the growth of chicks in the Gustafsson germ-free apparatus and in a conventional environment, with and without dietary supplements of penicillin," *British Journal of Nutrition* 17 (1963): 141–50.

which animals and why: The Pew Charitable Trust has focused on antibiotic use in food animals. In February 2013 it reported on record-high sales of antibiotics for meat and poultry production. It found that in 2011 nearly 80 percent (30 million) of the nearly 38 million pounds of antibiotics consumed each year in the United States was for animals sold for meat and poultry production. See http://www.pewhealth.org/other-resource/record-high-antibiotic-sales-for-meat-and-poultry-production-85899449119. See also a commentary by former FDA commissioner David Kessler, "Antibiotics and the meat we eat," *New York Times* op-ed page (March 27, 2013).

121 of 132 *Yersinia* samples: Consumer's Union tested 198 pork chops and ground-pork products purchased at retail in six U.S. cities. Of these, 69 percent were positive for *Yersinia enterocolitica*, an important foodborne pathogen that causes diarrheal and systemic illnesses, most of whose isolates were antibiotic resistant, and of those 39 percent were multiply resistant (*Consumers Reports*, January 2013).

bacteria resistant to antibiotics: 2011 Retail Meat Report from the National Antimicrobial Resistance Monitoring System. See it at: http://www.fda.gov/downloads/AnimalVeterinary/SafetyHealth/AntimicrobialRe

an indication of fecal contamination: The issues from the 2011 NARMS report are highlighted by the Environmental Working Group in its own report and analysis of the findings: D. Undurraga, "Superbugs invade American supermarkets," http://static.ewg.org/reports/2013/meateaters/ewg_meat_and_antibiotics_report2(

forbade the practice in 1999: M. Casewell et al., "The European ban on growth-promoting antibiotics and emerging consequences for human and animal health," *Journal of Antimicrobial Chemotherapy* 52 (2003): 159–61. The final ban on all growth-promoting antibiotics in the EU went into effect in 2006. But in some countries, farmers circumvented the ban with higher rates of treatments for "infections," which is allowed. Vigilance by regulators is needed.

patterns of antibiotic resistance: In fall of 2013 a large outbreak of *Salmonella heidelberg* from chickens was one of the latest episodes. The outbreak involved hundreds of people in more than twenty states. Many of the victims were hospitalized because of bloodstream infections caused by these multiply antibiotic-resistant organisms. See CDC, "Multistate outbreak of multidrug-resistant *Salmonella* heidelberg infections linked to Foster Farms brand chicken," http://www.cdc.gov/salmonella/heidelberg-10-13/index.html.

from contact with their animals: E. M. Harrison et al., "Whole genome sequencing identifies zoonotic transmission of MRSA isolates with the novel *mecA* homologue *mecC*," *EMBO Molecular Medicine* 5 (2013): 509–15.

especially sulfa drugs and tetracycline: In a November 1990 report to Congress, the General Accounting Office (GAO) indicated that twenty antibiotics were approved for use in dairy cows. It reported results of FDA testing of milk on retail store shelves in several surveys from 1988 to 1990. In all of them, antibiotics, particularly sulfa drugs (including sulfamethazine, which is not approved for use in cattle), were found. Reported rates ranged from 5 to 86 percent, and the GAO questioned whether the FDA tests were sufficiently sensitive. See GAO RCED 91-26, http://www.gao.gov/products/RCED-91-26 and http://www.gao.gov/assets/220/213321.pdf. In China, sulfas and quinolone antibiotics were detected in 40 percent and 100 percent, respectively, of milk sampled in 2011. Levels were reported as low but still widely present (R.-W. Han et al., "Survey of tetracyclines, sulfonamides, sulfamethazine, and quinolones in UHT milk in China market," *Journal of Integrative Agriculture* 12 [2013]: 1300–305).

from treatment plants, and tap water: C. Xi et al., "Prevalence of antibiotic resistance in drinking water treatment and distribution systems," *Applied and Environmental Microbiology* 75 (2009): 5714–18.

8 MOTHER AND CHILD

the toll of misery mounted relentlessly: Some doctors prescribed thalidomide to men for its sedative effects. It was safe because men absolutely could not get pregnant. One of these doctors was Jacob Sheskin, my grandmother's first cousin, a dermatologist who cared for patients with leprosy. When he gave thalidomide to several men with advanced leprosy to help them sleep, he observed that one type of their terrible skin lesions improved. He conducted careful clinical trials and proved to a skeptical world that this was true (J. Sheskin, "Thalidomide in the treatment of lepra reactions," *Clinical Pharmacology and Therapeutics* 6 [1965]: 303–6; and J. Sheskin, "The treatment of lepra reaction in lepromatous leprosy. Fifteen years' experience with thalidomide," *International Journal of Dermatology* 6 [1980]: 318–22). Sheskin was a clinician and did not understand the basis for thalidomide's action, but later others did, and they went on to extend its uses. Today thalidomide and a family of related drugs are used in cancer therapy as a mainstay for certain conditions, including multiple myeloma and other tumors. If someone predicted this fifty years ago, everyone would have assumed it to be a very sick form of joke.

Did not improve pregnancy outcomes in the least: From the early 1940s through the 1960s, diethylstilbestrol (DES) was prescribed for pregnant women to reduce the risk of pregnancy complications and losses. However, beginning in the early 1950s, studies began to appear in the obstetrics literature indicating that DES was not effective in promoting better pregnancy outcomes. For example, a widely cited clinical trial that was performed in Chicago showed no improvement in adverse pregnancy outcomes in women who were randomly assigned to receive DES or to serve as controls. (W. J. Dieckmann et al., "Does the administration of diethylstilbestrol during pregnancy have therapeutic value?" *American Journal of Obstetrics and Gynecology* 66 [1953]: 1062–81.) By the time that DES usage stopped in the late 1960s, millions of pregnant women (and thus their babies) had received the drug. See also R. J. Apfel and S. M. Fisher, *To Do No Harm: DES and the Dilemmas of Modern Medicine* (New Haven: Yale University Press, 1986).

clear-cell adenocarcinoma of the vagina: A. L. Herbst et al., "Adenocarcinoma of the vagina: association of maternal stilbestrol therapy with tumor appearance in young women," *New England Journal of Medicine* 284 (1971): 878–81.

(33.3 percent vs. 15.5 percent): R. Hoover et al., "Adverse health outcomes in women exposed in utero to diethylstilbestrol," *New England Journal of Medicine* 365 (2011): 1304–14. As indicated on its website, "the DES Follow-Up Study investigates the long-term health consequences associated with exposure to diethylstilbestrol (DES). Since 1992, the National Cancer Institute in collaboration with research centers throughout the United States has been conducting the DES Follow-Up Study of more than 21,000 mothers, daughters, and sons."

is coming under question: As discussed in the chapter 3 notes, recently investigators have pointed out that in many animal species, the transfer of microbes from mother to child begins before birth, while their baby is still in the womb (Funkhauser and Bordenstein, "Mom knows best"). There isn't much information yet about humans, but studies should address this in the next few years. If this occurs, then the importance of antibiotic use during pregnancy may rise.

women who have been studied: O. Koren et al., "Host remodeling of the gut microbiome and the metabolic changes during pregnancy," *Cell* 150 (2012): 470–80. This is the first part of the study in Ruth Ley's lab that is discussed below.

rapidly colonizes the mother's skin: M. G. Domínguez-Bello et al., "Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns," *Proceedings of the National Academy of Sciences* 107 (2010): 11971–75.

foundation of microbes: In a study of the gut microbiota of healthy people in three places—the United States, Malawi, and Venezuela (Amerindians)—Yatsunenko and her colleagues, including my wife, Gloria, catalogued which microbes were present across people of all ages (see chapter 1). Initially I was surprised, but the more I thought about it, the more sense it made. This was consistent with my hypotheses about the importance of the early-life microbiota.

the rate is about 4 percent: In Järna, a Swedish community near Stockholm, families try to sustain as natural a lifestyle as possible. They minimize antibiotic use, and virtually all of their babies are breast fed. They try to have C-sections only when absolutely required: their rate, at 4 percent, is lower than the rest of Sweden (about 17 percent), and a lot lower than that of the United States (32

percent). See J. S. Alm et al., “An anthroposophic lifestyle and intestinal microflora in infancy,” *Pediatric Allergy and Immunology* 13 (2002): 402–11.

to one in three births in 2011: In 1981, of one hundred women coming to the hospital to give birth, the rates in nineteen industrialized countries ranged from 5 percent in Czechoslovakia to 18 percent in the United States. (See F. C. Notzon et al., “Comparisons of national Cesarean-section rates,” *New England Journal of Medicine* 316 [1987]: 386–89.) More recently, the rates in the United States climbed to 30.5 percent between 2002 and 2008. (See J. Zhang et al., “Contemporary Cesarean delivery practice in the United States,” *American Journal of Obstetrics and Gynecology* 203 [2010]: 326.e1–10.) For births in 2011, the CDC reported a national rate of 32.8 percent, a more than 80 percent increase in thirty years from our already high rate.

13 percent in the Netherlands: According to the World Health Organization (2008), the highest rates in the world are in countries like Brazil (46 percent), Iran (42 percent), and the Dominican Republic (42 percent). The lowest country is the Netherlands (about 13 percent), and Scandinavia, in general, has rates much lower than that of the rest of the world. Is the medical care in Brazil, Iran, and the Dominican Republic more advanced than it is in northern Europe, or are other factors at play?

and all will get intravenous penicillin: Unless the mother is allergic to penicillin, in which case another antibiotic will be substituted.

acquired from his or her mother: “Prevention of Perinatal Group B Streptococcal Disease,” Revised Guidelines from CDC, 2010, MMWR, *Recommendations and Reports* 59(RR10): (Nov. 19, 2010): 1–32.

For our review of the subject of current antibiotic use in pregnancy, see W. J. Ledger and M. J. Blaser, “Are we using too many antibiotics during pregnancy?” *British Journal of Obstetrics and Gynecology* 120 (2013): 1450–52; I. A. Stafford et al., “Efficacy of maternal and neonatal chemoprophylaxis for early-onset group B streptococcal disease,” *Obstetrics and Gynecology* 120 (2012): 123–29. Although overall national rates of early-onset sepsis have diminished substantially, rates of early-onset GBS sepsis were unchanged after thirteen years of prophylaxis at one major medical center, reflecting a host of accumulated problems.

get the procedure: Episiotomy rates vary widely from country to country (see I. D. Graham et al., "Episiotomy rates around the world: an update," *Birth* 32 [2005]: 219–23). For an older, very comprehensive review, see G. Carroli and J. Belizan, "Episiotomy for vaginal birth," *Cochrane Database of Systematic Reviews* 3, no. CD000081 (2007): DOI: 10.1002/14651858.CD000081. Also see F. Althabe et al., "Episiotomy rates in primiparous women in Latin America: hospital-based descriptive study," *British Medical Journal* 324 (2002): 945–46.

first silver nitrate: Dr. Albert Barnes developed a dilute solution of silver nitrate around the turn of the twentieth century. It was called Argyrol and was used to treat gonorrheal eye infections that lead to blindness. He sold his company in 1929 for millions on the eve of the stock market crash. The proceeds from Argyrol formed the foundation for the renowned Barnes Foundation art collection in Philadelphia.

among the millions of births a year: We now screen pregnant women for HIV so that with proper prevention we can almost eliminate the risk of its transmission to the baby.

9. A FORGOTTEN WORLD

depending on context: Theodor Rosebury, a student and researcher of the oral microbiota beginning in the 1930s, had great insight into the biological relationships that we have with our residential organisms. His seminal works include: *Microorganisms Indigenous to Man* (New York: McGraw Hill, 1962) and *Life on Man* (London: Seeker and Warburg, 1969). In 1962 he coined the neologism *amphibiosis.* Today's scientists like the concept so much that they use it with a more modern name. They call the microbes pathobionts rather than the amphibionts originally described by Rosebury. But since it is the same idea, I give Rosebury credit and use his terms throughout.

in the continent's jungles and highlands: From Venezuelan patients who underwent upper gastrointestinal endoscopy, María Gloria Domínguez Bello (later my wife) and her colleagues in Venezuela obtained gastric biopsies from those living in urban areas near the coast and from deep in the interior in Puerto Ayacucho, the capital of Amazonas State. The *H. pylori* strains (that were isolated in pure culture by Chandra Ghose, a graduate student in my lab) from

the biopsies of some of the Amerindian patients in Puerto Ayacucho had genetic signatures that were closely related to strains from present-day people in China and Japan. In contrast, the strains isolated from the coastal patients had the signatures of present-day Europeans and Africans. The most parsimonious explanation for these findings is that the ancestors of the Amerindians had East Asian strains of *H. pylori* in their stomachs when they crossed the Bering Strait and that the descendants of those people and their *H. pylori* strains flourished until Columbus and the European conquest. With the decimation of the Amerindians and the introduction of *H. pylori* strains arriving in the stomachs of Europeans and their African slaves, there were few remaining Amerindian strains in coastal areas. But deep in the interior, these strains persisted, and the secret of their ancestry could be revealed by DNA sequencing. (See C. Ghose et al., "East Asian genotypes of *Helicobacter pylori* strains in Amerindians provide evidence for its ancient human carriage," *Proceedings of the National Academy of Sciences* 99 [2002]: 15107–11.) Subsequently, we worked with an international team that made sense of a worldwide collection of *H. pylori* isolates to understand how the organisms spread around the world in the past 58,000 years. (See D. Falush et al., "Traces of human migration in *Helicobacter pylori* populations," *Science* 299 [2003]: 1582–85.) In later studies, Gloria and her colleagues deciphered the whole genome sequence of one of the strains from Puerto Ayacucho, showing its uniqueness in the *H. pylori* universe. (See S. P. Mane et al., "Host-interactive genes in Amerindian *Helicobacter pylori* diverge from their old world homologs and mediate inflammatory responses," *Journal of Bacteriology* 192 [2010]: 3078–92.) **organisms from fecal specimens:** The methods used for isolation of *Campylobacter* were developed by Martin Skirrow, a clinical microbiologist in Worcester, England. It was his paper (M. Skirrow, "Campylobacter enteritis: a new disease," *British Medical Journal* 2 [1977]: 9–11), which I read in July 1977 shortly after I had been caring for the patient with *C. fetus* infection (see chapter 1), that brought me into the field of medical research. Later we modified Skirrow's medium (M. J. Blaser et al., "Campylobacter enteritis: clinical and epidemiologic features," *Annals of Internal Medicine* 91 [1979]: 179–85) to improve isolation of these fastidious organisms.

never bothered again by *H. pylori*: B. J. Marshall et al., "Attempt to fulfil Koch's postulates for pyloric campylobacter," *Medical Journal of Australia* 142 (1985): 436–39.

the same relationships in their own studies: B. J. Marshall et al., "Prospective double-blind trial of duodenal ulcer relapse after eradication of *Campylobacter pylori*," *Lancet* 2 (1988): 1437–42. An Irish group (J. G. Coghlan et al., "Campylobacter pylori and recurrence of duodenal ulcers—a 12-month follow-up study," *Lancet* 2 [1987]: 1109–11) published similar findings more than a year earlier but, despite more than five hundred citations, their paper was mostly forgotten, and Marshall *et al.* received most of the scientific credit. Later studies in the United States (D. Y. Graham et al., "Effect of treatment of *Helicobacter pylori* infection on the long-term recurrence of gastric or duodenal ulcer: a randomized, controlled study," *Annals of Internal Medicine* 116 [1992]: 705–8) and in Austria (E. Hentschel et al., "Effect of ranitidine and amoxicillin plus metronidazole on the eradication of *Helicobacter pylori* and the recurrence of duodenal ulcer," *New England Journal of Medicine* 328 [1993]: 308–12) cemented the finding that antibiotic treatments that eradicated *H. pylori* markedly improved the natural history of peptic ulcer disease, often leading to a cure.

based on their having antibodies to the organism: Guillermo received his doctoral degree based on studies we did together exploring the nature of the antigens of *C. jejuni* and *C. fetus* strains. By 1985 I was convinced that this new "campylobacter" could be medically important, so we began to apply the same biochemical and immunological approaches to it. (See G. I. Pérez-Pérez and M. J. Blaser, "Conservation and diversity of *Campylobacter pyloridis* major antigens," *Infection and Immunity* 55 [1987]: 1256–63; G. I. Pérez-Pérez et al., "*Campylobacter pylori* antibodies in humans," *Annals of Internal Medicine* 109 [1988]: 11–17.) **a very good lead:** We were particularly interested in comparing people with ulcers with those who had gastritis only. Among the seventy-four patients who had gastritis (meaning inflammation of the stomach), about 60 percent had antibodies to the CagA protein. But each of the thirty-one patients who had duodenal ulcers had these antibodies. (See T. L. Cover et al., "Characterization of and human serologic response to proteins in *Helicobacter pylori* broth culture supernatants with vacuolizing cytotoxin activity," *Infection and Immunity* 58 [1990]: 603–10.) For the first time, we had a blood test that could highlight people who were at high risk for getting ulcers. About a year and a half later, a group in England led by Jean Crabtree ("Mucosal IgA recognition of *Helicobacter pylori* 120 kDa protein, peptic ulceration, and gastric pathology," *Lancet* 338 [1991]: 332–35) identified the same protein with the

same proportions of people having antibodies when they had gastritis (~60 percent) and ulcers (100 percent) as we did. At that point, I was certain we had identified a critical *H. pylori* protein—two independent groups, across the ocean, with nearly identical observations—not a chance event. The confirmation makes a discovery a real discovery.

for cytotoxin-associated gene: As often happens in science, a second group, from the Biocene Company in Siena, Italy, was following a similar strategy. Even though we recognized the association with ulcers earlier and cloned the gene earlier, once they got started, they more rapidly uncovered many of the same associations. By chance we learned that they had identified the same gene and that they had given it a different name. Guided by a shared spirit of scientific collaboration, we eventually agreed on a common name, CagA, because those strains produced unusually high levels of the cytotoxin that injures human cells (cytotoxin-associated gene A). This collaboration saved the field from the uncertainty and divisiveness that occur when there are two names for the same thing. (See M. Tummuru et al., "Cloning and expression of a high-molecular-mass major antigen of *Helicobacter pylori:* evidence of linkage to cytotoxin production," *Infection and Immunity* 61 [1993]: 1799–809; A. Covacci et al., "Molecular characterization of the 128-kDa immunodominant antigen of *Helicobacter pylori* associated with cytotoxicity and duodenal ulcer," *Proceedings of the National Academy of Sciences* 90 [1993]: 5791–95.) **we discovered and named VacA:** T. L. Cover et al., "Divergence of genetic sequences for the vacuolating cytotoxin among *Helicobacter pylori* strains," *Journal of Biological Chemistry* 269 (1994): 10566–73; and T. L. Cover and M. J. Blaser, "Purification and characterization of the vacuolating toxin from *Helicobacter pylori*," *Journal of Biological Chemistry* 267 (1992): 10570–75, described the discovery of the protein, which we called VacA, and then we called the gene *vacA.* VacA was discovered as a toxin, but I now think of it as a signaling molecule, a way in which *H. pylori* tells the host what it wants the host to do. One effect of VacA is to tone down the immune response of T-cells, as a way of ensuring its own survival. (See B. Gebert et al., "*Helicobacter pylori* vacuolating cytotoxin inhibits T lymphocyte activation," *Science* 301 [2003]: 1099–1102.) If it is too toned down, there might not be enough inflammation for *H. pylori*, and thus not enough nutrients. So it has to strike a balance. Many years ago, Tim and I had the notion that CagA is the accelerator and VacA is the brake. It stills looks like a good idea.

But two years later: In 1989 we published an article in the *New England Journal of Medicine* on the relationship of *H. pylori* to gastritis (C. P. Dooley et al., "Prevalence of *Helicobacter pylori* infection and histologic gastritis in asymptomatic persons," *New England Journal of Medicine* 321 [1989]: 1562–66), again confirming the utility of our blood test. After he read that article, Dr. Nomura wrote to me. We communicated mostly by mail and sometimes by telephone. Although we worked closely on a number of studies that were very important to both of us, and worked very well together, we did not actually meet in person for about ten years!

strains had double the risk: Four papers all published in 1991 showed strong associations of having *H. pylori* and developing gastric cancer: J. Parsonnet et al., "*Helicobacter pylori* infection and the risk of gastric carcinoma," *New England Journal of Medicine* 325 (1991): 1127–31; A. Nomura et al., "*Helicobacter pylori* infection and gastric carcinoma among Japanese Americans in Hawaii," *New England Journal of Medicine* 325 (1991): 1132–36; D. Forman et al., "Association between infection with *Helicobacter pylori* and risk of gastric cancer: evidence from a prospective investigation," *British Medical Journal* 302 (1991): 1302–5; and N. J. Talley et al., "Gastric adenocarcinoma and *Helicobacter pylori* infection," *Journal of the National Cancer Institute* 83 (1991): 1734–39. Later we showed that having a *cagA*+ strain just about doubled the risk of gastric cancer development (M. J. Blaser et al., "Infection with *Helicobacter pylori* strains possessing *cagA* is associated with an increased risk of developing adenocarcinoma of the stomach," *Cancer Research* 55 [1995]: 2111–15) and of its precursor, chronic atrophic gastritis (E. J. Kuipers et al., "*Helicobacter pylori* and atrophic gastritis: importance of the cagA status," *Journal of the National Cancer Institute* 87 [1995]: 1777–80).

"the only good *Helicobacter* pylori is a dead one": D. Y. Graham, "The only good *Helicobacter pylori* is a dead *Helicobacter pylori*," *Lancet* 350 (1997): 70–71.

This ancient organism: Evidence that *H. pylori* is ancient: D. Falush et al., "Traces of human migration in *Helicobacter pylori* populations," *Science* 299 (2003): 1582–85; B. Linz et al., "An African origin for the intimate association between humans and *Helicobacter pylori*," *Nature* 445 (2007): 915–18; Y. Moodley et al., "The peopling of the Pacific from a bacterial perspective," *Science* 323 (2009): 527–30; S. Breurec et al., "Evolutionary history of

Helicobacter pylori sequences reflect past human migrations in Southeast Asia," *PLOS ONE* 6 (2011): e22058: 1–10; and Y. Moodley et al., "Age of the association between *Helicobacter pylori* and man," *PLOS Pathogens* 8 (2012): e1002693: 1–16.

whether his mother has it: J. Raymond et al., "Genetic and transmission analysis of *Helicobacter pylori* strains within a family," *Emerging Infectious Diseases* 10 (2004): 1816–21.

the human stomach has changed markedly: M. J. Blaser, "*Helicobacter pylori* eradication and its implications for the future," *Alimentary Pharmacology and Therapeutics* 11, suppl. 1 (1997): 103–7; "Not all *Helicobacter pylori* strains are created equal: should all be eliminated?" 349 *Lancet* (1997): 1020–22; "Helicobacters are indigenous to the human stomach: duodenal ulceration is due to changes in gastric microecology in the modern era," *Gut* 43 (1998): 721–27; "In a world of black and white, *Helicobacter pylori* is gray," *Annals of Internal Medicine* 130 (1999): 695–97.

they're safely across: M. J. Blaser and D. Kirschner, "The equilibria that allow bacterial persistence in human hosts," *Nature* 449 (2007): 843–49.

10. HEARTBURN

have symptoms every day: G. M. Eisen et al., "The relationship between gastroesophageal reflux and its complications with Barrett's esophagus," *American Journal of Gastroenterology* 92 (1997): 27–31; and H. B. El-Serag, "Time trends of gastroesophageal reflux disease: a systematic review," *Clinical Gastroenterology and Hepatology* 5 (2007): 17–26.

progress to the form of cancer called adenocarcinoma: J. Lagergren et al., "Symptomatic gastroesophageal reflux as a risk factor for esophageal adenocarcinoma," *New England Journal of Medicine* 340 (1999): 825–31.

first identified in 1950: In 1950, Norman Barrett, an English surgeon, reported finding abnormal tissue in the esophagus. We now call that abnormality Barrett's esophagus, and he became Sir Norman Barrett.

in the past three decades: The incidence of adenocarcinoma of the esophagus is

rising, and not just because of better surveillance and reporting. (See H. Pohl and H. G. Welsh, "The role of overdiagnosis and reclassification in the marked increase of esophageal adenocarcinoma incidence," *Journal of the National Cancer Institute* 97 [2005]: 142–46.) **eight times more likely:** J. J. Vicari et al., "The seroprevalence of *cagA*-positive *Helicobacter pylori* strains in the spectrum of gastroesophageal reflux disease," *Gastroenterology* 115 (1998): 50–57; and M. F. Vaezi et al., "*CagA*-positive strains of *Helicobacter pylori* may protect against Barrett's esophagus," *American Journal of Gastroenterology* 95 (2000): 2206–11. Other more recent GERD/Barrett's studies include: D. Corley et al., "*Helicobacter pylori* infection and the risk of Barrett's oesophagus: a community-based study," *Gut* 57 (2008): 727–33; and L. A. Anderson et al., "Relationship between *Helicobacter pylori* infection and gastric atrophy and the stages of the oesophageal inflammation, metaplasia, adenocarcinoma sequence: results from the FINBAR case-control study," *Gut* 57 (2008): 734–39. All show inverse associations, with the strongest data from the Corley study showing that people with *cagA*+ strains have a 92 percent reduction in the risk of developing Barrett's esophagus.

double the rate of esophageal disease: J. Labenz et al., "Curing *Helicobacter pylori* infection in patients with duodenal ulcer may provoke reflux esophagitis," *Gastroenterology* 112 (1997): 1442–47.

colleagues from around the world: W. H. Chow et al., "An inverse relation between *cagA*$^+$ strains of *Helicobacter pylori* infection and risk of esophageal and gastric cardia adenocarcinoma," *Cancer Research* 58 (1998): 588–90; R. Peek et al., "The role of *Helicobacter pylori cagA*$^+$ strains and specific host immune responses on the development of premalignant and malignant lesions of the gastric cardia," *International Journal of Cancer* 82 (1999): 520–24; R. J. L. F. Loffeld et al., "Colonization with *cagA*-positive *H. pylori* strains inversely associated with reflux oesophagitis and Barrett's oesophagitis," *Digestion* 62 (2000): 95–99; and F. Kamangar et al., "Opposing risks of gastric cardia and noncardia gastric adenocarcinomas associated with *Helicobacter pylori* seropositivity," *Journal of the National Cancer Institute* 98 (2006): 1445–52.

and are more damaging: Two studies that were presented at scientific congresses in the late 1990s were very instructive. In the Eurogast Study (P. M. Webb et al., "Gastric cancer, cytotoxin-associated gene A-positive *Helicobacter pylori*, and serum pepsinogens: an international study," *Gastroenterology* 116

[1999]: 269–76), 2,850 patients in thirteen countries who underwent upper gastrointestinal endoscopy were examined with stomach biopsies, and assessed for blood levels of proteins that are made in the stomach. As was by then expected, people with *H. pylori* had a higher rate of showing a blood-protein (pepsinogen) ratio indicative of atrophic changes than those without the organism, and those with *cagA*+ strains had even more altered blood-protein ratios than those with *cagA*-negative strains. In a contemporaneous study, Y. Yamaji *et al.* ("Inverse background of *Helicobacter pylori* antibody and pepsinogen in reflux oesophagitis compared with gastric cancer: analysis of 5732 Japanese subjects," *Gut* 49 [2001]: 335–40) showed that in Japan people who had reflux had a pattern of gastric tissue changes and protein production that was the inverse of those with stomach cancer. As the signs of atrophic gastritis—a precursor to gastric cancer—rose, the prevalence of reflux fell. These two studies, done with thousands of patients, provide support for the mixed roles of *H. pylori* vis-à-vis diseases of the stomach and the esophagus.

11. TROUBLE BREATHING

we understood how these strains operate: Mechanism of action in CagA+ strains. In 1995 we published the first evidence that *H. pylori* had a Type-IV secretion system that could transport *H. pylori* products into the cells lining the stomach wall, but we did not know what was being exported (M. Tummuru et al., *Helicobacter pylori picB*, a homologue of the *Bordetella pertussis* toxin secretion protein, is required for induction of IL-8 in gastric epithelial cells," *Molecular Microbiology* 18 [1995]: 867–76). By 2000, several groups (most notably S. Odenbreit et al., "Translocation of *Helicobacter pylori* CagA into gastric epithelial cells by Type IV secretion," *Science* 287 [2000]: 1497–1500; as well as A. Covacci and R. Rappuoli, "Tyrosine-phosphorylated bacterial proteins: Trojan horses for the host cell," *Journal of Experimental Medicine* 191 [2000]: 587–92) proved that there was a Type-IV system, and that the material it injected was none other than the CagA protein that we (and Covacci) had found a decade earlier, in our case, probing the library of *H. pylori* genes with my serum (see chapter 9). No wonder that I made antibodies to CagA; the *H. pylori* strain that I was carrying was injecting the protein into my stomach wall every day for years.

at the age of seven: A. L. Kozyrskyj et al., "Increased risk of childhood asthma from antibiotic use in early life," *Chest* 131 (2007): 1753–59.

in May of that year: M. E. Fernández-Beros, L. Rogers, G. I. Pérez-Pérez, W. Hoerning, M. J. Blaser, and J. Reibman, "Seroprevalence of *Helicobacter pylori* is associated with later age of onset of asthma in urban adults," abstract presented in May 2005 at the American Thoracic Society Annual Meeting in San Diego, CA.

the subject's *H. pylori* status: In the late 1990s Guillermo had run tests on more than eleven thousand people in that study as part of a government contract. He had just received little numbered tubes of serum without any knowledge of any of the characteristics of the people they came from. He was completely blinded, and some of the samples were in fact deliberate duplicates to see how reproducible the assays were. His results were beautifully reproducible, which made the sponsors (and us) very pleased. After the paper was published (J. E. Everhart et al., "Seroprevalence and ethnic differences in *Helicobacter pylori* infection among adults in the United States," *Journal of Infectious Diseases* 181 [2000]: 1359–63), eventually a lot of the data from NHANES III became publicly available in complicated tables and spreadsheets that qualified statisticians could examine. We had earlier examined the relationship of *H. pylori* and obesity using NHANES III (I. Cho et al., "*Helicobacter pylori* and overweight status in the United States: data from the Third National Health and Nutrition Examination Survey," *American Journal of Epidemiology* 162 [2005]: 579–84) and did not find any relationship, but we had practice using the complex NHANES III data set.

my hypothesis was correct: J. Reibman et al., "Asthma is inversely associated with *Helicobacter pylori* status in an urban population," *PLOS ONE* 3 (2008): e4060: 1–6; and Y. Chen and M. J. Blaser, "Inverse associations of *Helicobacter pylori* with asthma and allergies," *Archives of Internal Medicine* 167 (2007): 821–27.

studies show consistent results: Y. Chen and M. J. Blaser, "*Helicobacter pylori* colonization is inversely associated with childhood asthma," *Journal of Infectious Diseases* 198 (2008): 553–60.

the modern *H. pylori*–free stomach: R. Rad et al., "CD25+/Foxp3+ T cells regulate gastric inflammation and *Helicobacter pylori* colonization in vivo,"

Gastroenterology 131 (2006): 525–37; and K. Robinson et al., "*Helicobacter pylori*–induced peptic ulcer disease is associated with inadequate regulatory T cell responses," *Gut* 57 (2008): 1375–85.

as Mueller and her colleagues showed in mice: I. C. Arnold et al., "*Helicobacter pylori* infection prevents allergic asthma in mouse models through the induction of regulatory T cells," *Journal of Clinical Investigation* 121 (2011): 3088–93; and M. Oertli et al., "DC-derived IL-18 drives Treg differentiation, murine *Helicobacter pylori*–specific immune tolerance, and asthma protection," *Journal of Clinical Investigation* 122 (2012): 1082–96.

in the next twenty-one years: A. Nomura et al., "*Helicobacter pylori* infection and the risk for duodenal and gastric ulceration," *Annals of Internal Medicine* 120 (1994): 977–81.

in the other organisms present and their distribution: M. J. Blaser, "Helicobacters are indigenous to the human stomach: duodenal ulceration is due to changes in gastric microecology in the modern era," *Gut* 43 (1998): 721–27.

12. TALLER

one of my colleagues at NYU: Lewis Goldfrank, MD, chair of the Department of Emergency Medicine at NYU Langone Medical Center and at Bellevue Hospital Center.

a rural community in Guatemala: L. Mata, *The Children of Santa Maria Cauque: a Prospective Field Study of Health and Growth* (Cambridge, MA: MIT Press, 1978).

the development of adult height: A. S. Beard and M. J. Blaser, "The ecology of height: the effect of microbial transmission on human height," *Perspectives in Biology and Medicine* 45 (2002): 475–98.

acquired in the first few years of life: Even today, we don't fully know how *H. pylori* is acquired in early life. We know that having an *H. pylori*–negative mother strongly reduces the risk of a child acquiring the organism, but thus far it has not been found in the vagina, and even in communities in which nearly all of the mothers are positive, we rarely detect it in their children before the age of

one year. Either it was there all along and was suppressed or it is actually acquired later, still from the mother or from siblings, father, or friends (in day care and school). Or it is possible that for one hundred positive children, it is a mixture of all of these routes, but it remains a mystery. We know that it is not from the family dog, because dogs don't carry *H. pylori*; they have their own helicobacters.

the same fecal-oral route: This is a mechanism for transmitting microbes in the feces of one person into the mouth of another. Food, water, hands may be intermediaries. Infectious diseases such as polio, hepatitis A, and typhoid fever are transmitted this way.

the hormones ghrelin and leptin: C. U. Nwokolo et al., "Plasma ghrelin following cure of *Helicobacter pylori*," *Gut* 52 (2003): 637–40; and F. François et al., "The effect of *H. pylori* eradication on meal-associated changes in plasma ghrelin and leptin," *BMC Gastroenterology* 11 (2011): 37.

keeping records of it: Beard and Blaser, "The ecology of height."

bad things can happen: M. J. Blaser and D. Kirschner. "The equilibria that allow bacterial persistence in human hosts," *Nature* 449 (2007): 843–49.

13. … AND FATTER

visiting scholars from around the world: But where did the money come from to do the work? There is a paradox in the way that medical science is funded in the United States and elsewhere. In order to get a grant, you must have "preliminary data" that support the idea to be tested, to see whether it may be feasible. But how do you do the feasibility studies without the money that the grant provides? It is a catch-22. I was fortunate at the time to have the means to fund a new idea. First, because I had a number of ongoing research projects, I had amassed over the years equipment and supplies that we could use for a new project. Then, being at a university, there were students and trainees who were looking for a new project, a way to develop their own career track. Ilseung came my way for that reason. I also had received support from several philanthropists that was not exactly earmarked; I could spend it at my discretion. We often say that discretionary money is worth double because of its flexibility. With that support, I could make a commitment to Laurie when she was looking for a lab in which to do graduate work. Finally, there is luck, too. A colleague told me about

a neighbor who was looking to work in the lab for a summer. He also told me that she was a student at Princeton. I felt that was a good predictor that she would have something on the ball, and when I met Yael that premise was immediately confirmed. In the United States, the conduct of science is very entrepreneurial, and after good ideas hard work is the sine qua non of success.

"What's happening to their body composition?": Ilseung later applied for and received an NIH-supported grant from NYU's Clinical and Translational Science Institute (CTSI) that allowed him to conduct this research. CTSI director Dr. Bruce Cronstein, who was a member of a mentoring committee to help Ilseung solve research problems, has studied the metabolism of bone for many years and had a DEXA machine that he used for his own mouse studies. He made the suggestion that opened up new vistas for us. In science, it also "takes a village" to get things done.

the adipose tissue, where fat is stored: I. Cho et al., "Antibiotics in early life alter the murine colonic microbiome and adiposity," *Nature* 488 (2012): 621–26. This was Ilseung's major work in the lab and involved him and twelve other scientists—biochemists, animal experimenters, informaticists, gene expression analysts—each contributing to a different aspect of the work. But Ilseung's patient studies and the sixteen months we spent conducting new experiments and clarifying our work for the anonymous reviewers and the editors of *Nature* paid off, and the paper was finally published, more than five years after the work had started.

But when they are conventionalized: The germ-free state is artificial; there are no germ-free animals except within specialized laboratories. When germ-free animals have their microbiota restored, it is said that they have been "conventionalized" back to the usual (natural) state.

from the normal-weight mouse donor: In Jeff's experiments, the germ-free mice can be used as living test tubes that can react to the newly introduced microbiota. ("Germ-free" means that the playing field is even and clean, tabula rasa.) (P. Turnbaugh et al., "A core gut microbiome in obese and lean twins," *Nature* 457 [2009]: 480–84.) **can make a big difference:** Laurie later did studies to assess how faithfully we had transferred the microbes from their original hosts to the recipients. The DNA sequencing results showed that we did amazingly well. Even microbes for which we had the evidence of only a single sequence in our snapshot of what was there were well represented in the

recipients. Thus we had confidence that the germ-free mice were colonized with what actually was in the STAT or control mice. Interestingly, the community of STAT microbes didn't do as well in their new hosts as the community of untreated microbes. The population was less resilient, and were less resistant to invasion by new species. In this second generation of STAT mice, the microbiota were punier, and I worry about this. See chapter 15, "Antibiotic Winter."

all the antibiotics prescribed for American children: We decided to study the two classes of antibiotics most commonly used for human children. The first, beta-lactams, include penicillin, amoxicillin, Augmentin (amoxicillin with a second compound to inhibit bacterial enzymes that would inactivate it), and cephalosporins. Amoxicillin is the number-one drug prescribed to young children in the United States and in most developed countries. In 2010 there were nearly 23 million courses of amoxicillin or Augmentin prescribed to children in the United States and more than 6.5 million of those courses were for children under the age of two. (G. Chai et al., "Trends of outpatient prescription drug utilization in U.S. children, 2002–2010," *Pediatrics* 130 [2012]: 23–31.) That averages to nearly one course of those amoxicillin-based antibiotics per young child per year. Macrolides are the second class of antibiotics used for young children. The best known is erythromycin, available for more than fifty years, but in the past twenty years, longer-acting and broader-spectrum agents have been used, including clarithromycin and azithromycin (the Z-pak, which has benefited from a marketing strategy as good as any ever employed). In 2010 U.S. children received more than 10 million courses of azithromycin, which has become the most widely prescribed antibiotic in the United States. It was so expensive that anyone who bought it was likely to use it. (It is now off-patent and the price has dropped.) The tylosin that we used is the macrolide that can most easily and inexpensively be used in mice and for which there is an extensive literature that helped us figure out the correct doses.

the drug that most promotes the recent increases in human height: The increase in human height began before antibiotics were discovered, at least in the Western countries. But these experiments (both STAT and PAT) indicate that antibiotics—and it is not limited to a single type—affect microbiome composition (see below) and can affect early-life bone development. Certainly this could be part of the story and could explain why the recent height increases in China are recapitulating in forty years the increases that took one hundred

years in Europe and the United States.

colleagues at Washington University in St. Louis: We worked closely together with Drs. Erica Sodergren and George Weinstock, who run a major genome-sequencing center at Washington University in St. Louis. Once we received the sequence information from them, Alex Alekseyenko, an NYU faculty member who is an expert in bioinformatics, decoded and deconstructed the data, and then analyzed it.

passed on to them by their mother: We could not find evidence for the presence of many of the microbes seen in the mothers and in the control mice. Either they had been permanently eliminated or they were still present but in low numbers, below our ability to detect them—in which case, the bacteria that bloomed under the influence of the tylosin regimen were still suppressing them—long after the tylosin was gone. This can happen because they get such an advantage in early life—a "founder effect"—that they are able to sustain their increased numbers.

big studies now under way: Through Dr. Ernst Kuipers, my former postdoctoral trainee and now longtime friend, we were working with a group in the Netherlands to address this. A large cohort, including more than ten thousand mothers and their newborn children in Rotterdam, have been enrolled in the kind of study that could provide answers to important questions in development, but it will take several years for the kids to get old enough to have any reasonable outcome data. The United States is in the early stages of the National Children's study, whose goal is to enroll up to one hundred thousand children, get lots of information, and see what kind of outcomes—especially asthma, obesity, and diabetes—they develop. The results of that study also will become available years from now.

Avon Longitudinal Study of Parents and Children (ALSPAC) study in Britain: Drs. Leo Trasande and Jan Blustein, NYU faculty members who work primarily in pediatrics and health policy, respectively, are expert epidemiologists. They both found the ALSPAC Study (J. Golding et al., "ALSPAC—the Avon Longitudinal Study of Parents and Children, I. Study methodology," *Paediatric and Perinatal Epidemiology* 15 [2001]: 74–87) and led the analyses (L. Trasande et al., "Infant antibiotic exposures and early-life body mass," *International Journal of Obesity* 37 [2013]: 16–23; J. Blustein et al., "Association of caesarian delivery with child adiposity from age 6 weeks to

15 years," *International Journal of Obesity* 37 [2013]: 900–906).

may also contribute to the risk: A Boston study of 1,255 mother-child pairs (S. Y. Huh et al., "Delivery by caesarean section and risk of obesity in preschool children: a prospective cohort study," *Archives of the Diseases of Childhood* 97 [2012]: 610–16) found a significantly increased obesity risk in offspring born by C-section. A Canadian study (K. Flemming et al., "The association between caesarean section and childhood obesity revisited: a cohort study," *Archives of the Diseases of Childhood* 98 [2013]: 526–32) showed that C-section was a risk factor overall, but when they controlled for excessive maternal weight it dropped out. Similarly in our ALSPAC study (J. Blustein et al.), nearly all of the risk was in babies born of mothers who already were overweight. There are many potential explanations for this; one is that the overweight mothers already have a depleted microbiota and C-section adds to the problem in the next generation. In Brazil, where in 2009 the C-section rate went above 50 percent, meaning that more than half the 3 million births in that country were by C-section, two studies showed different results. H. A. S. Goldani *et al.* ("Cesarean delivery is associated with an increased risk of obesity in adulthood in a Brazilian birth cohort study," *American Journal of Clinical Nutrition* 93 [2011]: 1344–47), studying a 1978 birth cohort twenty-three to twenty-five years later, found about a 50 percent increase in obesity in C-section babies that could not be explained by other factors. But F. C. Barros *et al.* ("Cesarean section and risk of obesity in childhood, adolescence, and early adulthood: evidence from 3 Brazilian birth cohorts," *American Journal of Clinical Nutrition* 95 [2012]: 465–70), studying three later birth cohorts, showed effects in the same direction, but they were not statistically significant. The authors discussed unaccounted confounding factors in their study. However, in a later study by the same authors (B. L. Horta et al., "Birth by Caesarean Section and Prevalence of Risk Factors for Non-Communicable Diseases in Young Adults: A Birth Cohort Study," *PLOS ONE* 8 [2013]: e74301), a follow-up of the 1982 birth cohort to the time of their entrance exam into the army at age eighteen (and with follow-up to age twenty-three), they found that C-section was associated with increased body mass index (BMI), amount of body fat, and also systolic blood pressure.

the relationship of C-section with these other modern plagues: Other disease risks for which some studies have shown increased risk with C-sections: A. K. Hansen et al., "Risk of respiratory morbidity in term infants delivered by elective caesarean section: cohort study," *British Medical Journal* 336 (2008): 85–87; C.

Roduit et al., “Asthma at 8 years of age in children born by caesarean section,” *Thorax* 64 (2008): 107–13; H. Renz-Polster et al., “Caesarean section delivery and the risk of allergic disorders in childhood,” *Clinical and Experimental Allergy* 35 (2005): 1466–72; P. Bager et al., “Caesarean delivery and risk of atopy and allergic disease: meta-analyses,” *Clinical and Experimental Allergy* 38 (2008) 634–42; and C. R. Cardwell et al., “Caesarean section is associated with an increased risk of childhood-onset type 1 diabetes mellitus: a meta-analysis of observational studies,” *Diabetologia* 51 (2008): 726–35. Not every study shows an association with these diseases; some studies are small and underpowered, and others have multiple confounding factors, but there certainly is mounting evidence that the biological costs of C-section are experienced not just during the first month after birth.

14. MODERN PLAGUES REVISITED

disappearing before their second birthdays: Type-1 DM epidemiology is changing in the United States: T. H. Lipman et al., “Increasing incidence of type 1 diabetes in youth. Twenty years of the Philadelphia Pediatric Diabetes Registry,” *Diabetes Care* 36 (2013): 1597–1603; and in Europe: C. C. Patterson et al., “Incidence trends for childhood type 1 diabetes in Europe during 1989–2003 and predicted new cases 2005–2020: a multicenter prospective registration study,” *Lancet* 373 (2009): 2027–33.

babies who gain weight more rapidly in the first year of life: E. Bonifacio et al., “Cesarean section and interferon-induced helicase gene polymorphisms combine to increase childhood type 1 diabetes risk,” *Diabetes* 60 (2011): 3300–306; R. M. Viner et al., “Childhood body mass index (BMI), breastfeeding and risk of Type 1 diabetes: findings from a longitudinal national birth cohort,” *Diabetic Medicine* 25 (2008): 1056–61; M. Ljungkrantz et al., “Type 1 diabetes: increased height and weight gains in early childhood,” *Pediatric Diabetes* 9 (2008): 50–56; and E. Hypponen et al., “Obesity, increased linear growth, and risk of type 1 diabetes in children,” *Diabetes Care* 23 (2000): 1755–60. In classic studies of migrants to England, Bodansky and colleagues showed that the children born in the new place (UK) developed higher rates than those born in the old country (H. J. Bodansky et al., “Evidence for an environmental effect in the aetiology of insulin dependent diabetes in a transmigratory population,”

British Medical Journal 304 [1992]: 1020–22). Together, all of these point to strong environmental influences driving the increased rates of Type 1 diabetes, but farm exposure does not appear to be important (K. Radon et al., "Exposure to farming environments in early life and type 1 diabetes: a case-control study," *Diabetes* 54 [2005]: 3212–16).

But could anything accelerate it?: NOD (non-obese diabetic) mice are a particular strain of mice that have enhanced susceptibility to developing autoimmune diabetes, with many characteristics that resemble Type 1 diabetes in human children. Affected mice have progressive immune-mediated destruction of the Islets of Langerhans, where insulin is produced. The strain was first recognized in Japan in the late 1970s. (See H. Kikutani and S. Makino, "The murine autoimmune diabetes model: NOD and related strains," *Advances in Immunolology* 51 [1992]: 285–322.) Diabetes develops in 50–80 percent of female mice and 20–40 percent of males. Interestingly, when mice are kept in spanking-clean cages and rooms, they get more diabetes. In rooms with a lot of shared bedding, the rates go down. The general observation is that "dirty protects." This suggests that there are transmissible agents (microbes) whose presence affects the risk of diabetes development. The sex difference in the NOD mice differs from that of humans but permits analyses of the relevant factors underlying the dichotomy.

allowed us to include both in the experiments: Ali and I discussed this. Despite the JDRF limitation, we agreed that we would study both PAT and STAT because we wanted two chances for success. Fortunately I had some other funds from a philanthropist that I could use for the additional expenses, and Ali had received support from the Howard Hughes Medical Institute to advance her career. And with the promise of funding from JDRF, I said to Ali, "Your preliminary work is going so well, instead of just taking one year off from medical school, why don't you take more time off and get a PhD for your work." This would entail a big change in her career plans. I suggested this to her on a Friday. By Monday, she had decided. "I am going for it!" she excitedly told me, and the NYU MD/PhD program immediately accepted her. She has been an incredible student, already making important discoveries.

more than quadrupling since 1950: Celiac disease is rising in incidence: T. Not et al., "Celiac disease risk in the USA: high prevalence of antiendomysium antibodies in healthy blood donors," *Scandinavian Journal of Gastroenterology*

33 (1998): 494–98. One in 250 healthy blood donors in the United States: P. H. R. Green et al., "Characteristics of adult celiac disease in the USA: results of a national survey," *American Journal of Gastroenterology* 96 (2001): 126–31. One in 133 adults; 1 in 56, if you include related disorders: J. F. Ludvigsson et al., "Increasing incidence of celiac disease in a North American population," *American Journal of Gastroenterology* 108 (2013): 818–24. One in 141, based on NHANES data: A. Rubio-Tapia, "The prevalence of celiac disease in the United States," *American Journal of Gastroenterology* 107 (2012): 1538–44.

compared to those who didn't: K. Marild et al., "Antibiotic exposure and the development of coeliac disease: a nationwide case-control study," *BMC Gastroenterology* 13 (2013): 109.

led by Dr. Ben Lebwohl at Columbia University: B. Lebwohl et al., "Decreased risk of celiac disease in patients with *Helicobacter pylori* colonization," *American Journal of Epidemiology* 178 (2013): 1721–30.

people born by C-section also face an increased risk: K. Marild et al., "Pregnancy outcome and risk of celiac disease in offspring: a nationwide case-control study," *Gastroenterology* 142 (2012): 39–45.

the risk of developing IBD at an early age: A. Hviid et al., "Antibiotic use and inflammatory bowel diseases in childhood," *Gut* 60 (2011): 49–54.

in the first year of life: A. L. Kozyrskyj et al., "Increased risk of childhood asthma from antibiotic use in early life," *Chest* 131 (2007): 1753–59.

as many as one in fifty children has the condition: S. H. Sicherer et al., "US prevalence of self-reported peanut, tree nut, and sesame allergy: 11-year follow-up," *Journal of Allergy and Clinical Immunology* 125 (2010): 1322–26.

I was able to find records: L. Hicks et al., "US outpatient antibiotic prescribing, 2010," *New England Journal of Medicine* 368 (2013): 1461–62.

just for those under two: G. Chai et al., "Trends of outpatient prescription drug utilization in US children, 2002–2010," *Pediatrics* 130 (2012): 23–31.

highest in the states with the highest obesity: The CDC data on macrolides were first revealed at a meeting (L. Hicks et al., "Antimicrobial prescription data reveal wide geographic variability in antimicrobial use in the United States,

2009," presented at the forty-eighth annual meeting of the Infectious Disease Society of America, Vancouver, Canada, October 21–24, 2010), and the abstract is available online at https://idsa.confex.com/idsa/2010/webprogram/Paper3571.html. In addition to total antibiotic use, the scientists examined both macrolide use and fluoroquinolone use. Fluoroquinolones include ciprofloxacin, levofloxacin, and others. All three maps—total use, macrolides, and fluoroquinolones—look very similar.

I am focusing on macrolides because fluoroquinolones are not often used for children, whereas macrolides are. Azithromycin was the number-two most-prescribed antibiotic in U.S. children in 2010 (see G. Chai et al., "Trends of outpatient prescription drug utilization in US children"). The CDC data do not distinguish between which macrolides are used in which state, but azithromycin is likely to dominate everywhere because of its remarkable growth in sales. A final caveat is that the CDC maps show antibiotic use across all ages. It is not broken down by year of life, so we do not know whether the relationships observed across all ages also hold for children. Such analyses should be done. Obesity levels source: "Overweight and Obesity" (Atlanta: Centers for Disease Control, 2012), accessed http://www.cdc.gov/obesity/data/adult.html.

one in eighty-eight children has autism or autism spectrum disorder: The generally accepted first written observation of a problem with autism was the paper by Austria-born Leo Kanner, who had established a child psychiatry clinic at Johns Hopkins Hospital (L. Kanner, "Autistic disturbances of affective contact," *Nervous Child* 2 [1943]: 217–50). Since its recognition, there has been much evidence for its rise, despite a trend toward overdiagnosis of autism and its related disorders. See I. Hertz-Picciotto and L. Delwiche, "The rise in autism and the role of age at diagnosis," *Epidemiology* 20 (2009): 84–90; C. J. Newschaffer et al., "The epidemiology of autism spectrum disorders," *Annual Review of Public Health* 28 (2007): 235–58. In 2012, the Centers for Disease Control released an estimate that one in eighty-eight children has an autism-spectrum disorder (http://www.cdc.gov/media/releases/2012/p0329_autism_disorder.html).

can affect cognitive development and mood: Rodent studies of gut signaling to the brain, involving the microbiome: J. F. Cryan and T. G. Dinan, "Mind-altering micro-organisms: the impact of the gut microbiota on brain and

behavior," *Nature Reviews Neuroscience* 13 (2012): 701–12; and R. Diaz Heijtz et al., "Normal gut microbiota modulates brain development and behavior," *Proceedings of the National Academy of Sciences* 108 (2011): 3047–52.

the blood of autistic children: D. Kiser et al., "Review: the reciprocal interaction between serotonin and social behavior," *Neuroscience & Biobehavioral Reviews* 36 (2012): 786–98; and B. O. Yildirim and J. J. L. Derksen, "Systematic review, structural analysis and a new theoretical perspective on the role of serotonin and associated genes in the etiology of psychopathology and sociopathy," *Neuroscience & Biobehavioral Reviews* 37 (2013): 1254–96.

have important bearing on our estrogen status: We have reviewed this topic (C. S. Plottel and M. J. Blaser, "Microbiome and malignancy," *Cell Host & Microbe* 10 [2011]: 324–35), which includes many of the primary references.

it's not their genes: M. C. King et al., "Breast and ovarian cancer risks due to inherited mutations in BRCA1 and BRCA2," *Science* 302 (2003): 643–46. According to the U.S. National Cancer Institute, about 12 percent of U.S. women develop breast cancer at some time in their life, but women who have mutations in BRCA1 have a 55 to 65 percent chance, and those with mutations in BRCA2 have a 45 percent chance of developing the cancer before the age of seventy. Ovarian cancer is less common, but having a BRCA mutation increases the risk even more (lifetime risk in the general population is 1.4 percent; the risk with BRCA1 is 39 percent and the risk with BRCA2 is 11–17 percent). Dr. Mary Claire King was one of the discoverers of BRCA1 and has been a pioneer in studies to understand its significance. In her 2003 review in *Science*, she provided data indicating that women who have these mutations develop breast cancers at different ages. However, what was particularly alarming to me is that among women born after 1940, the age curve had shifted to the left. At any given age, women with BRCA1 or BRCA2 mutations who were born after 1940 had a much higher risk of developing breast cancer than women born before 1940. Although comprehensive genetic analyses were not done, such data suggest that there has been a strong environmental risk added to the genetic risk seen in these women.

as we speculated five years ago: M. J. Blaser and S. Falkow, "What are the consequences of the disappearing human microbiota?" *Nature Reviews Microbiology* 7 (2009): 887–94.

brilliant *Silent Spring*: Rachel Carson, *Silent Spring* (New York: Houghton Mifflin, 1962). I read it when I was thirteen. It greatly affected my thinking about the interconnectedness on our planet.

15. ANTIBIOTIC WINTER

a fifty-six-year-old Brooklyn native: Peggy Lillis's family have started the Peggy Lillis Memorial Foundation to promote public education about *C. diff*.

A recent study of nearly 2 million: R. E. Polk et al., "Measurement of adult antibacterial drug use in 130 US hospitals: comparison of defined daily dose and days of therapy," *Clinical Infectious Diseases* 44 (2007): 664–70.

lead to greater toxin production: V. G. Loo et al., "A predominantly clonal multi-institutional outbreak of *Clostridium difficile*–associated diarrhea with high morbidity and mortality," *New England Journal of Medicine* 353 (2005): 2442–49; and M. Warny et al., "Toxin production by an emerging strain of *Clostridium difficile* associated with outbreaks of severe disease in North America and Europe," *Lancet* 366 (2005): 1079–84.

overall picture of drug-resistant bacteria in the United States: "CDC Threat Report 2013: Antibiotic resistance threats in the United States, 2013," at http://www.cdc.gov/drugresistance/threat-report-2013/.

fending off disease-causing bacteria: In their initial experiments, Marjorie Bohnhoff and her colleagues showed that the dose of *Salmonella* required to infect half of the exposed mice went from about 100,000 bacterial cells to 3, following a one-day exposure to the antibiotic streptomycin. (M. Bohnhoff et al., "Effect of streptomycin on susceptibility of intestinal tract to experimental *Salmonella* infection," *Proceedings of the Society for Experimental Biology and Medicine* 86 [1954]: 132–37.) In later studies, the team extended the work, showing that penicillin was just as effective as streptomycin, that they could enhance susceptibility of mice to a *Staphylococcus* species that was incapable of colonizing by itself, and that injecting the antibiotic into tissues had no effect, thus implicating the normal gut bacteria in the protective effect and their depletion by antibiotics in promoting infections. (M. Bohnhoff and C. P. Miller, "Enhanced susceptibility to *Salmonella* infection in streptomycin-treated mice," *Journal of Infectious Diseases* 111 [1962]: 117–27.) These and further

observations are more than fifty years old, but they have been largely forgotten.

160,000 people became ill and several died: C. Ryan et al., "Massive outbreak of antimicrobial-resistant salmonellosis traced to pasteurized milk," *Journal of the American Medical Association* 258 (1987): 3269–74.

found in the human gut and on human skin: M. Sjölund et al., "Long-term persistence of resistant *Enterococcus* species after antibiotics to eradicate *Helicobacter pylori*," *Annals of Internal Medicine* 139 (2003): 483–87; and M. Sjölund et al., "Persistence of resistant *Staphylococcus epidermidis* after a single course of clarithromycin," *Emerging Infectious Diseases* 11 (2005): 1389–93. *Staphylococcus epidermidis* is a very common type of *Staphylococcus* that colonizes the human skin, and it has much less potential to be a pathogen than *S. aureus*. Change in its abundance is a good indicator of perturbations of the skin environment.

a large number of much less common ones: Fundamental studies have been done in the last few years describing the outlines of the populations of residential bacteria in our bodies, as well as the genes they carry. For an introduction into this area, see C. Huttenhower et al., "Structure, function and diversity of the healthy human microbiome," *Nature* 486 (2012): 207–14; and J. Qin et al., "A human gut microbial gene catalogue established by metagenomic sequencing," *Nature* 464 (2010): 59–64.

microbial diversity and the genes that accompany it: T. Yatsunenko *et al.* found that adults in the United States carried 15–25 percent fewer bacterial species in their intestines than did people who were from Malawi, or were Amerindians in Venezuela, respectively (T. Yatsunenko et al., "Human gut microbiome viewed across age and geography," *Nature* 486 [2012]: 222–27). Le Chattlier and colleagues found that a large proportion of Europeans had about 40 percent fewer bacterial genes than Europeans with a full complement of genes. Those with low gene numbers were much more likely to be obese (E. Le Chatelier et al., "Richness of human gut microbiome correlates with metabolic markers," *Nature* 500 [2013]: 541–46). Although these data are consistent with our idea that depletion of our resident microbes predisposes to obesity (M. J. Blaser and S. Falkow, "What are the consequences of the disappearing human microbiota?" *Nature Reviews Microbiology* 7 [2009]: 887–94), the data do not yet permit ascertaining the direction of causality.

16. SOLUTIONS

I recommended that she start antibiotics immediately: Lyme disease is caused by *Borrelia burgdorferi*, a bacterium that lives mostly in rodents but can be transmitted by ticks to larger mammals like deer and us.

it kills bacteria on contact: Triclosan, an antimicrobial and antifungal agent, has been used since the late 1960s to prevent hospital-acquired infections. It was put into underarm deodorants in the 1970s to reduce microbial populations that contribute to human body smells. Today triclosan is in thousands of products: soaps, toothpaste, pizza cutters, mouthwash, clothing, cleaning supplies, mattresses, and some flooring—anywhere you might want to reduce bacterial or fungal counts. You also see little dispensers of hand sanitizers not only in hospitals but also in grocery stores, offices, classrooms, conference centers, hotels, gyms, in fact, everywhere. As advertisers vilify germs, the public slathers on the triclosan and many products with similar antibacterial effects. Evidence that triclosan is affecting the bacterial communities that live on us is growing. See S. Skovgaard et al., "*Staphylococcus epidermidis* isolated in 1965 are more susceptible to triclosan than current isolates," *PLOS ONE* 16 (2013): e62197; D. J. Stickler and G. L. Jones, "Reduced susceptibility of *Proteus mirabilis* to triclosan," *Antimicrobial Agents and Chemotherapy* 52 (2008): 991–94; and A. E. Aiello et al., "Relationship between triclosan and susceptibilities of bacteria isolated from hands in the community," *Antimicrobial Agents and Chemotherapy* 48 (2004): 2973–79.

are being prescribed annually to U.S. children: G. Chai et al., "Trends of outpatient prescription drug utilization in U.S. children, 2002–2010," *Pediatrics* 130 (2012): 23–31. Of the top eight drugs given to U.S. children in 2010, five were antibiotics, accounting for more than 41 million individual courses. In steady state, just these five antibiotics would account for about ten courses per child during the first eighteen years of life, and the evidence suggests that we have improved in recent years, so the rate was probably higher in the past. Four of the five are beta-lactam antibiotics, the descendants in a sense of pencillin, and the other was azithromycin, the "Z-pak." Interestingly, the other three drugs in the top-eight list, accounting for 13 million courses, are mostly used for asthma (see chapter 11).

people living in western states: L. Hicks et al., "US outpatient antibiotic prescribing, 2010," *New England Journal of Medicine* 368 (2013): 1461–62.

the highest rate of antibiotic use: O. Cars et al., "Variation in antibiotic use in the European Union," *Lancet* 357 (2001): 1851–53. France had more than fourfold higher use than nearby Netherlands.

"only when necessary": V. Blanc et al., "'Antibiotics only when necessary' campaign in the Alpes-Maritimes District: no negative impact on invasive infections in children in the community 1998–2003," *Presse Med* 37 (2008): 1739–45. Use has fallen by about half (B. Dunais et al., "Antibiotic prescriptions in French day-care centres: 1999–2008," *Archives of Disease in Childhood* 96 [2011]: 1033–37).

204 **And in Sweden:** In response to the U.S. study, Swedish investigators summarized their country's antibiotic use in 2012. The differences are striking. Not only is the aggregate usage less than half (47 percent) of ours, but in the first three years of life, the most crucial period, on average Swedish children are receiving less than one and a half courses of antibiotics versus about four in U.S. children. We are not seeing higher death rates in Swedish children (in fact they are lower), nor more hearing deficits. The regional variation is also less, the difference between the extremes of urban Stockholm (408/1000) and the rural north (315/1000) about 30 percent. See A. Ternhag and J. Hellman, "More on U.S. outpatient antibiotic prescribing, 2010," *New England Journal of Medicine* 369 (2013): 1175–76. These numbers tell us that major reductions in prescribing can be readily accomplished.

for patients with cancer: My dad had a low-grade lymphoma diagnosed in his late eighties. He did well on no treatment until about five years later, when he developed a severe form of anemia. Now treatment was needed. After receiving an antibody for a protein on the surface of his malignant cells, he responded immediately and well. In total, he received four weekly injections while sitting in a chair watching TV for a few hours each time. The treatment was fantastic, but the cost, $110,000, was huge. He needed three more of these courses over the next couple of years, and now, nearly five years later, he is fine. He paid his insurance premiums all of those years and made his contributions to Social Security as well. Treatment with this designer drug definitely extended both the quality and quantity of his life. Pharmaceutical companies and hospitals can make large returns this way as long as insurance plans still pay. He has a

relatively uncommon condition. But to treat millions of infections in young children with designer drugs of the kind I outlined would break the bank; a different economic is needed.

by identifying specific agents: X. Hu et al., "Gene expression profiles in febrile children with defined viral and bacterial infection," *Proceedings of the National Academy of Science*s 110 (2013): 12792–97.

which organism is causing the trouble: A. Zaas et al., "A host-based RT-PCR gene expression signature to identify acute respiratory viral infection," *Science Translational Medicine* 5 (2013): 203ra126.

even higher than it is in the United States: L. Dong, "Antibiotic prescribing patterns in village health clinics across 10 provinces of Western China," *Journal of Antimicrobial Chemotherapy* 62 (2008): 410–15. Hospitals can mark up the price of antibiotics sold to patients, providing financial incentives for their overuse. One estimate is that Chinese patients have more than double the antibiotic use of U.S. patients, and on pig farms, it is four times more. In a survey of large pig farms Y.-G. Zhu *et al.* found 149 different antibiotic-resistance genes, often at extremely high concentrations (Y.-G. Zhu et al., "Diverse and abundant antibiotic resistance genes in Chinese swine farms," *Proceedings of the National Academy of Sciences* 110 [2013]: 3435–40).

no one really knows what causes it: Diverticulitis is a complication of diverticulosis, a condition with finger-size or smaller out-pouching in the colon. Usually, diverticulosis has no symptoms, and is mostly associated with aging, but occasionally it leads to diverticulitis. As in the patient described, it can be a painful illness with fever, due to inflammation of the wall of the out-pouch.

a much stronger scientific base for their efficacy: A few probiotics have been successful in treating or preventing infectious diseases. We have limited evidence that probiotics can help prevent *C. diff* infection, and possibly protect against serious infections due to the particularly virulent *E. coli* (O157:H7) strains (K. Eaton et al., "A cocktail of non-pathogenic bacteria naturally occurring in the digestive tract of healthy humans can protect against a potentially lethal *E. coli* infection [EHEC 0157:H7]," abstract presented at the 113th Annual Meeting of the American Society of Microbiology, Denver, CO, May 2013). Eaton and her colleagues gave EHEC to mice colonized by six normal human commensals of the gut or not colonized at all and found that there

was no toxin production in the former group but high levels in the latter. These findings suggest possible probiotic candidates for the prevention or treatment of serious EHEC infections.

pivotal and attention-getting study: Solid evidence that fecal transplantation works to cure patients with recurrent *C. diff* infections (E. van Nood et al., "Duodenal infusion of donor feces for recurrent *Clostridium difficile*," *New England Journal of Medicine* 368 [2013]: 407–15).

not far-fetched to think: R. A. Koeth et al., "Intestinal microbiota metabolism of L-carnitine, a nutrient in red meat, promotes atherosclerosis," *Nature Medicine* 19 (2013): 576–85; W. H. W. Tang et al., "Intestinal microbial metabolism of phosphatidylcholine and cardiovascular risk," *New England Journal of Medicine* 368 (2013): 1575–84.

ruling was quite reasonable: A group of physicians and scientists (including myself) was asked by the American Gastroenterological Association to comment on the ruling. Our consensus was that it was appropriate, and we discussed the reasoning and implications. See G. Hecht et al., "What is the value of an FDA IND for fecal microbiota transplantation to treat *Clostridium difficile* infection?" *Clinical Gastroenterology and Hepatology* (2014), in press.

can teach us the key principles?: I. Pantoja-Feliciano, "Biphasic assembly of the murine intestinal microbiota during early development," *ISME Journal* 7 (2013): 1112–15. Ida, who studied for her PhD with Gloria, examined the relationship of the microbiota in mice in relation to their mother's vagina and intestine. In earliest life, the gut organisms of the pups looked like those of their mother's vagina. While they were nursing, they had a very restricted microbiota dominated by a few major bacteria, like lactobacillus, and then after they were weaned, the profile changed again and resembled that of their mother's intestine. In a few short weeks, Gloria's group had recapitulated the early-life development of the intestinal residents of human children.

Rob Knight and José Clemente: Rob Knight, a very tall, thin biochemist originally from New Zealand who heads a large research group in Colorado, has been brilliant in the creation of software programs to analyze the complexity of the microbiome and to deconvolute it. José Clemente, originally from Spain, came to work with Rob via Japan and now has his own lab in New York. Rob had come in on a conference and was staying with us. José took the subway

down. I was getting ready to take the train up to Brown to speak about my own work. Still I couldn't resist listening in on them, and witnessing the discoveries for myself.

back to our children: M. J. Blaser, "Science, medicine, and the future: *Helicobacter pylori* and gastric diseases," *British Medical Journal* 316 (1998): 1507–10.

EPILOGUE

ice cap in Greenland would melt: For a comprehensive view of global warming for the nonscientist, see, for example: B. E. Johansen, *The Encyclopedia of Global Warming Science and Technology*, vols. 1 and 2 (Santa Barbara, California: Greenwood Publishing, 2009); and for some solutions: M. Z. Jacobson and M. A. Dilucchi, “A path to sustainable energy by 2030,” *Scientific American* 301 (2009): 58–65.

INDEX

The index that appeared in the print version of this title does not match the pages in your e-book. Please use the search function on your e-reading device to search for terms of interest. For your reference, the terms that appear in the print index are listed below.

A Beautiful Mind abscesses
acid
    reflux
    stomach
    -suppressive therapies adaptive immunity
adenocarcinoma
adrenaline
agriculture
    antibiotics used in
    growth promotion
    invention of
algae
allergies

antibiotics and
food
gluten
H. pylori and penicillin
skin test
Allobaculum ALSPAC study
American Thoracic Society amino acids
amoxicillin
amphibiosis
ampicillin
anaerobic bacteria
ancestral populations
anemia
animals
antibiotics used in
as pathogen hosts
Antarctica
antibiotics
agricultural use
antibiotic winter
asthma and
autism and
brain development and
broad-spectrum
celiac disease and
development of
diabetes and
discovery of penicillin early-life use and effects estrogen and
in food
food allergies and
growth-promoting
heartburn and
height and
IBD and
narrow-spectrum
in newborns
obesity and
overuse of

overuse of
in pregnancy and birth prescription rates
reduced use of
resistance. *See* resistance, antibiotic side effects
solutions
see also specific antibiotics antigens
aphids
archaea arsenic
asthma
antibiotics and
childhood
GERD and
H. pylori and Atherton, John
Atlanta
atomic bomb
atopic dermatitis
Augmentin
Australia
autism
antibiotics and
azithromycin

bacteria
antibiotic winter and
in basalt
beneficial
colonization
consortia
C-section and
development of antibiotics and distinction between virus and diversity
dual nature of *H. pylori* ecosystems
evolution of
food and
genes
gut
height and
immune system and
loss of friendly bacteria marine

microbial world
newborns and
obesity and
overuse of antibiotics pathogens
pregnancy and
probiotics
resistant
transfer during childbirth *see also* microbes; *specific bacteria and infections*
bacteriophage
Bacteroides Bangladesh
Barrett's esophagus
Beard, Albertine
Benz, Karl
Bering Strait
beta-lactams
Bifidobacter biofilms
birds
birth defects
Bitterroot Valley, Montana Black Death, plague in Europe Blaser, Genia
blood pressure
bloodstream
glucose
infections
Blustein, Jan
body mass index (BMI)
Bohnhoff, Marjorie
bone
infections
brain
role of gut microbes in early development Brandt, Lawrence
Brazil
BRCA genes
breast cancer
breast development
breast milk
breath, bad
broad-spectrum drugs
Brussels

Brussels
Buchnera C. diff infections fecal microbiota transfer CagA
Campylobacter cancer
antibiotics and
BRCA genes and
DES and
stomach, and *H. pylori see also specific cancers* carbapenem-resistant enterobacteriaceae (CRE) cecum
celiac disease
antibiotics and
H. pylori and Centers for Disease Control and Prevention (CDC) Cesarian section
diabetes and
obesity and
overreliance on
Chain, Ernst
cheating
chemotherapy
Chen, Yu
chickenpox
childbirth
antibiotic use in
Cesarean section
episiotomy
fever
microbe transfer during children
acid reflux
antibiotics overused in asthma
autistic
death rates
DES and thalidomide
diabetes
early-life antibiotic use and effects *H. pylori* in height
high-fat diet

IBD

microbe transfer during childbirth microbiome of newborns and bacteria
obesity
reduced use of antibiotics in rise of illness in China
chloramphenicol
chlorination
Cho, Ilseung
cholera
cholesterol
ciprofloxacin
Civil War
clarithromycin
Clemente, Jose
Cleveland Clinic
climate
change
clindamycin
Clostridium Clostridium difficile (*C. diff*) infection cognition
colon
C. diff infection cancer
colostrum
commensalism
competition
contingency microbes
Cooley, Fred
cooperation
co-trimoxazole
cough
Cox, Laurie

CRE

Crohn’s disease
crowd diseases

Darwin, Charles

Darwin, Charles
death
    cancer
Deinococcus radiodurans, and radioactivity *Delftia acidovorans*, in gold mining
dendritic cells
Denmark
dentists
DES (diethylstilbestrol) DEXA scans
diabetes
    adult-onset (type 2)
    antibiotics and
    C-section and
    gestational
    juvenile
diagnostics
    distinction between viruses and bacterial infections diarrheal illnesses
digestion
digoxin
diphtheria
diversity, microbial
    loss of
diverticulitis

DNA

doctors
    C-section rates
    malpractice
    prescription rates
    responsible prescription of antibiotics salaries
Domagk, Gerhard
Dominguez Bello, Maria Gloria doxycycline
Dunne, Jessica
DuraSTAT
dysentery

E. coli ear infections

Ebola
ecosystems, microbial
eczema
Ehrlich, Paul
encephalitis
England
Engstrand, Lars
Enterococcus faecalis, *faecium* enzymes
epidemics
episiotomy
erythromycin
esophagus
    acid reflux
    cancer
estrobolome
estrogen
    antibiotics and
eukaryotes
Europe
evolution
extinctions
extremophiles
eye infections, in newborns family size
Faroe Islands
FatSTAT
fecal contamination
fecal microbiota transplantation (FMI) Feigenbaum, Rardi
fermentation
fever
fiber
Finland, diabetes in
Firmicutes Fleming, Alexander
Florey, Howard
fluoroquinolones
food
    allergies
    antibiotics in
    baby

baby
digestion
gluten-free
high-fat
improved nutrition
lack of
microbes and
organic
Food and Drug Administration (FDA) Ford, Henry
France
Frieden, Tom
fungi

game theory
gangliosides
gastritis
H. pylori and GCLO. *See Helicobacter pylori* genes
ancient
microbial
variant
genomics
geology
GERD (Gastroesophageal reflux disease) asthma and
Germany
germ-free mice
germ theory
Ghana, obesity in
ghrelin
Giardia
gluten allergy
Gondwana
gonorrhea
Gordon, Jeff
Gould, Stephen Jay
Greenland, ice cap in
growth promotion
Guatamala, Santa Maria Cauque Guerrero, Isabel
gut

bacteria
-brain interactions
infections
loss of friendly bacteria normal flora
probiotics
role of bacteria in pregnancy *Haemophilus influenzae* Hantavirus
Hawaii
hay fever
heart
disease
valve infections
heartburn
height
antibiotics and
growth spurt
H. pylori and increase in
microbes and
Helicobacter pylori asthma and
celiac disease and
disappearance of
discovered as a pathogen dual nature of
esophageal disease and gastritis and
height and
immunity and
proteins
reintroduction of, into children stomach (gastric) cancer and transmission
ulcers and
Heliobacter species, other than *pylori* hepatitis
herpes virus
herpes zoster
high blood pressure
HIV/AIDS
Homo sapiens Hoover, Robert
horizontal transmission hormones
estrogen and antibiotics hospitals
C-section rates
infections
profits

profits
hosts
animals as
Human Microbiome Project (HMP) hunter-gatherers
hygiene hypothesis

immunity
adaptive
development of
H. pylori and hygiene hypothesis
innate
microbial
India
Industrial Revolution
infectious diseases
antibiotic-resistant
antibiotic winter and
development of antibiotics epidemic
modern plagues
pathogens and
see also specific diseases Infectious Diseases Society of America (IDSA)
infertility
inflammation
beneficial
H. pylori and stomach
inflammatory bowel disease (IBD) antibiotics and
influenza
innate immunity
insects
insulin
insurance
intestines
C. diff infection

IBD

ulcers

see also gut Inuit
irritable bowel syndrome isoniazid
Italy

Japan

Kanner, Leo
Kelsey, Francis
Kessler, David
keystone species
Khoruts, Alexander
kidneys
kissing
Knight, Rob
Koch, Robert
Komodo dragon

La Benz, Joachim
lactobacilli
Lactobacillus Lanetti, Ricky
latency
Laurasia
Lebwohl, Ben
leptin
Ley, Ruth
Lillis, Peggy
Lister, Joseph
Livanos, Alexandra
liver
cancer
Ludvigsson, Jonas
lungs
asthma
cancer
Lyme disease
lymphocytes
lysozyme

macrolides
malaria
malpractice
Manitoba, Canada
Marburg virus
Marianas Trench
Marild, Karl
Marmor, Michael
Marshall, Barry
mastoiditis
Mata, Leonardo
McPhee, John
measles
meconium
Mediterranean diet
memory
meningitis
menstruation
metabolism
    effect of antibiotics on MetaHit consortium
metronidazole
Mexico
microbes
    antibiotic winter and
    consortia
    C-section and
    diversity
    dual nature of *H. pylori* ecosystems
    epidemic
    evolution of
    fecal microbiota transplantation (FMT) food and
    genes
    gut
    height and
    human
    immune system and
    marine
    obesity and

obesity and
pathogens
resistant
terminology
transfer during childbirth world of
see also bacteria; *specific microbes* microbiome
disappearance hypothesis human
milk
breast
Miller, Anne
Miller, C. Phillip
molds
Penicillium mosquitoes
mouse studies
STAT experiments
mouth

MRSA

mucus
Mueller, Anne

narrow-spectrum antibiotics Nash, John
National Cancer Institute (NCI) National Institutes of Health (NIH) natural selection
nervous system
Netherlands
New Guinea, tribes in
New World
New York University
NHANES (National Health and Nutrition Examination Survey) nitrogen
nitromidazoles
Nobel, Brandon
Nobel, Yael
Nobel Prize
Nomura, Abraham

normal flora nose
nurses
nut allergies

Obama, Barack
obesity
    antibiotics and
    childhood
    C-section and
    STAT experiments
oceans
odors, microbial
oligosaccharides
oral contraceptives
ovarian cancer
Oxalobacter oxytetracycline
oxytocin

pancreas, and diabetes panspermia hypothesis
parasites
paratyphoid
Pasteur, Louis
PAT experiments
pathogens
    antibiotic-resistant
    antibiotic winter and
    epidemic
    H. pylori discovered as latency
    population growth and
    as predators
    rise of
    see also specific pathogens pathology
Peek, Richard
Peggy Lillis Memorial Foundation penicillin
    allergy
    childhood obesity and
    discovery of

first use of
in pregnancy and birth resistance to
Penicillium molds Peoria, Illinois
Pérez-Pérez, Guillermo
peristalsis
peritoneum
Perlino, Carl
phages
pharmaceutical companies “broad-spectrum” approach failure to develop new antibiotics growth promotion and
H. pylori and profits
pine beetle
placebo effect
plague
Plasmodium falciparum plastic
Plottel, Claudia
pneumococcus
pneumonia
polio
pollution
population growth
prebiotics
pregnancy
antibiotic used in
bacteria and
DES and thalidomide used in diabetes and
probiotics
prokaryotes
prontosil, first sulfa drug proteins
H. pylori protists
public health
puerperal sepsis

Quammen, David

rabies
Reibman, Joan
reptiles

replies
resistance, antibiotic
ancient nature of
antibiotic winter
C. diff infections failure to develop new antibiotics growth promotion and

MRSA

Salmonella respiratory infections
Revolutionary War
rheumatic fever
rickettsia
Rocky Mountain spotted fever Rosebury, Theodore
rubella
rumen bacteria

Salmonella Salmonella typhi Salvarsan
sanitation
sanitizers

SARS

scarlet fever
Schwarz, Dragutin
Semmelweis, Ignatz
serotonin
sex
Sheskin, David
shingles
short-chain fatty acids (SCFA) silver nitrate
sinus infections
skeletons
skin bacteria
childbirth and
Skirrow, Martin
smallpox

soil
solutions
    diagnostics
    fecal microbiota transfer narrow-spectrum drugs
    probiotics
    reduced prescription of antibiotics restoring missing microbes Soper, George
spinal tap
Stanford, Leland, Jr.
Staph infections *Staphylococcus aureus Staphylococcus epidermidis* STAT experiments
stomach
    acid
    cancer, and *H. pylori H. pylori* in inflammation
strep infections
strep throat
streptococci, including Group B
Streptococcus pneumoniae streptomycin
sulfa drugs
sulfonamide
superbugs
surgical infections
survival of the fittest symbiosis
synbiotics
syphilis

Taft, William Howard
T-cells
teeth
testosterone
tetracycline
thalidomide
throat infections
TransSTAT
Trasande, Leo
T-reg cells
triclosan
Trinidad, bubbling tar lake tuberculosis
turista (Montezuma's revenge) tylosin

turista (Montezuma's revenge) tylosin
typhoid
Typhoid Mary (Mary Mallon) typhus

ulcerative colitis
ulcers
    H. pylori and University of Colorado Medical Center urban growth
urea
urinary tract infections VA (Veterans Affairs) hospital VacA
vaccines
vagina
    bacteria
    birth
    cancer
    episiotomy
    microbe transfer during childbirth Vanderbilt University
varicella-zoster
Venezuela
vernix
vertical transmission
viridans streptococci
viruses
    distinction between bacteria and epidemics
    hosts
    latent
    pathogens
    see also specific viruses vitamin B_{12}
vitamin D deficiency
vitamin K
vomiting

Warren, Robin
Washington, George
water
    antibiotics in
    contamination
West Virginia
wheat allergy

whooping cough
wolves
World Health Organization (WHO) World War I
World War II

Yale
yaws
yeast infections
Yellowstone National Park *Yersinia* Yukon permafrost

Zaire
Z-pak

ACKNOWLEDGMENTS

Writing, as with science, often takes a village, especially when the author, like me, has a different day job. I am much indebted to my daughter Simone Blaser for helping me shape my early ideas into a form that could be attractive to a publisher, and to Dorian Karchmar, my agent (and Simone's boss at William Morris), who helped me get there. Sandra Blakeslee did yeoman work converting my ideas and prose, generated by an academic, into a manuscript that could be more widely understood. To Sandra, with her endless creativity, intellect, and energy, and who I now count as one of the most important teachers in my career, I will be forever grateful. Gillian Blake, editor in chief at Henry Holt, and an enthusiast about this work from the very start, contributed in too many ways to count, and I learned that with regard to both style and content she was always right.

Many of my colleagues read portions of the manuscript to help determine whether or not I was on track and accurate. I appreciate the efforts of Drs. William Ledger, Ernst Kuipers, Claudia Plottel, and José

Clemente, and the important suggestions of Erika Goldman. Dr. Robert Anderson read the work as both physician and reader, and he gave great advice. I am indebted to Dr. Jan Vilcek for his critical insights as well; although English is not his native language, Jan also corrected my grammar. Linda Peters and Isabel Teitler helped me understand what could be understood and was interesting. I appreciate the friendship they each shared, helping me to craft this manuscript. My assistants at New York University, Sandra Fiorelli, Jessica Stangel, and then Joyce Ying, helped make order from chaos, no small feat, and I am most appreciative of their efforts. Adriana Pericchi Dominguez was an assiduous and resourceful fact-checker.

An important segment of the book focuses on the research done in my lab at Vanderbilt University and, over the past fourteen years, at NYU. At Vanderbilt, Drs. Tim Cover, Murali Tummuru, Guillermo Pérez-Pérez, Richard Peek, John Atherton, and Ernst Kuipers played key roles. At NYU, it also was very much a team effort, involving other faculty members, graduate and medical students, college and high school students, and visiting researchers. So many were involved in substantive ways that it would difficult to name them all. But for the work highlighted in the text, Drs. Guillermo Pérez-Pérez, Zhiheng Pei, Fritz Francois, Joan Reibman, Yu Chen, Zhan Gao, Ilseung Cho, Claudia Plottel, Alex

Alekseyenko, Leo Trasande, and Jan Blustein—all fellow NYU faculty members—contributed in ways mentioned and not. I have had outstanding graduate students and postdoctoral fellows who worked with me on the experiments discussed, notably Laurie Cox, Shingo Yamanishi, Alexandra Livanos, Sabine Kienesberger, and Victoria Ruiz. Yael Noble worked as a research assistant before her time in medical school, but in her efforts she was more like a grad student. Many other students, postdocs, and colleagues are working on ongoing projects that one day will be described in great detail in original scientific publications. Together we have had and continue to have an amazing lab, with a great culture of sharing and generosity.

Hurricane Sandy hit us very hard. With a loss of electrical power, we had a mad dash to retrieve our thawing specimens in freezers—the work of thirty years of research. We rescued nearly all of the current studies, but lost some of our archives—samples obtained from villages and patients all over the world decades ago. They were irreplaceable. We were out of our home lab at the New York Veterans Affairs hospital for more than ten months and had one tribulation piled on the next. Yet with their kindness to one another, adaptability, and “can do” mentality, it was, for the lab members, their finest hour, and the storm and its aftermath provided lessons in life that can not be learned from books.

For the past eight years, my research has had major philanthropic support in the form of the Diane Belfer Program in Human Microbial Ecology. Diane was an early believer in the value of our studies. I much appreciate her enthusiasm and unwavering support, beginning when the ideas were more of a dream. Early support also came from the Ellison Medical Foundation. More recently, the Knapp Family Foundation and the Leslie and Daniel Ziff Foundation have been major sponsors of our explorations. Our work also has been supported by the D'Agostino Foundation, Hemmerdinger Foundation, Fritz and Adelaide Kaufman Foundation, Margaret Q. Landenberger Research Foundation, Graham Family Charitable Foundation, James and Patricia Cayne Trust, and Messrs. David Fox, Richard Sharfman, Michael Saperstein, Robert Spass, and Joseph Curcio, and Dr. Bernard Levine, as well as Mss. Regina Skyer, Edythe Heyman, and Lorraine DiPaolo. Donna Marino has been an incredibly effective advocate for our work. I am very grateful to all.

Our work described in this book has been supported by funding from the National Institutes of Health, the U.S. Army, the Department of Veterans Affairs, the Juvenile Diabetes Research Foundation, the Howard Hughes Medical Institute, the Bill and Melinda Gates Foundation, the Robert Wood Johnson Foundation, the Ellison Medical Foundation, the International Union

against Cancer, the World Health Organization, and governments and universities in Japan, the Netherlands, Korea, United Kingdom, Switzerland, Finland, Sweden, France, Italy, Turkey, and Venezuela for support of visiting scholars. Institutional support came in many different forms from the NYU Langone Medical Center and from the Manhattan/NY Harbor Department of Veterans Affairs Medical Center.

This combination of major research university, U.S. government, private foundations, international support, and philanthropy is necessary for a research program to survive and ultimately to flower.

Finally, my wife and research partner, Dr. Maria Gloria Domínguez Bello, has helped with insight, criticism, adventure, and love. I am glad that I could highlight a few of her many contributions to our shared field. My children Daniel, Genia, and Simone have been steadfast in their love and support.

As with most projects that take a long time, many hands stirred the pot and contributed greatly. I thank one and all for their wonderful help and fellowship.

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MISSING MICROBES: HOW THE OVERUSE OF ANTIBIOTICS IS FUELING OUR MODERN PLAGUES

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Jacket art based on original image © iStock
The Library of Congress has cataloged the print edition as follows: Blaser, Martin J.

Missing microbes: how the overuse of antibiotics is fueling our modern plagues / Dr. Martin Blaser.

pages cm Includes index.

ISBN 978-0-8050-9810-5 (hardback) — ISBN 978-0-8050-9811-2 (electronic copy) 1. Antibiotics. 2. Antibiotics—Effectiveness. 3. Drug resistance in microorganisms. I. Title.

RM267.B57 2014

615.7'922—dc23 2013042578

First Edition: April 2014