

THE
INHABITED
BODY

When the Partnership Breaks



Dr Horst Herb

BACTERIA · ARCHAEA · EUKARYA

The Inhabited Body — Volume 2: Feeding, Fixing, and Fighting Your Microbiome

What We Do to Our Microbiome — and What It Does Back

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Chapter 18: Antibiotics — Collateral Damage

18.1 The Broad-Spectrum Problem: Carpet-Bombing an Ecosystem

On the evening of 11 December 1945, Alexander Fleming stood at a lectern in Stockholm to deliver his Nobel Lecture. He had already said what the audience came to hear — the story of the contaminated Petri dish, the ring of inhibition around the *Penicillium* colony, the miracle drug that had just finished saving Allied soldiers from wound infections in Europe and the Pacific. But he ended his lecture with a warning that the audience, exhausted by war and intoxicated by the promise of antibiotics, was in no mood to take seriously.

“It is not difficult,” Fleming said, “to make microbes resistant to penicillin in the laboratory by exposing them to concentrations not sufficient to kill them, and the same thing has occasionally happened in the body.” He went on to describe, with a kind of clinical premonition, how a man who bought penicillin at the chemist’s and took it for a sore throat might give his streptococci enough exposure to select for resistant mutants, then pass those mutants to his wife, who might die of a resistant pneumonia — “and who is responsible for that lady’s death? Why, the man who negligently played with penicillin” [REF:fleming1945].

It was the first public warning about antibiotic resistance, delivered by the discoverer himself, in the year of penicillin’s greatest triumph. It was ignored. For the next seventy years, antibiotics were prescribed as if the supply were infinite, the pathogens static, and the ecosystem they passed through empty.

That ecosystem, of course, was not empty. By the time a course of amoxicillin reaches the small bowel, it enters a community of hundreds of bacterial species, living in structured relationships with the mucus layer, with each other, and with the host. The drug does not distinguish between the pathogen the physician was worrying about and the commensals the physician never thought about. It kills what it can reach, in the concentrations it can reach, and it leaves the survivors to fill the vacancies. What happens next is the subject of this chapter.

The Ecologist’s Objection

If an ecologist were asked to describe modern antibiotic therapy in ecological terms, they would probably reach for a metaphor from forestry. Imagine a mature, old-growth forest — centuries in the making, with thousands of species arranged in layered niches, each holding the others in check through competition, predation, and mutualism. Now imagine treating that forest for a bark-beetle outbreak by aerial spraying with a broad-spectrum herbicide. You would certainly kill the bark beetles’ host trees, and thereby the beetles, but you would also kill most of the other trees, most of the understory, most of the fungi, most of the insects, and — indirectly — most of the animals that depend on any of these. The bark-beetle problem would be solved. The forest would be gone.

This is not a strained analogy. When the oncologist Martin Blaser, working at New York University, began asking in the mid-2000s whether the antibiotic era had been quietly producing an ecological catastrophe inside human bodies, the forest metaphor was one he returned to repeatedly [REF:blaser2014]. His argument, developed over a decade of research and eventually published as the book *Missing Microbes*, was that broad-spectrum antibiotics do not merely treat an infection —

they depopulate a habitat, and the recolonisation that follows is not always complete. Some species come back. Some do not. The forest that regrows is not the forest that was there before.

The empirical question behind Blaser's hypothesis is simple: when you finish a course of antibiotics, does your microbiome really return to baseline? For decades, the assumption was yes. Bowel flora were thought to be resilient, self-restoring, and more or less interchangeable between individuals. The few studies that looked at the question used culture methods that could see only a fraction of the community, and they generally reported that things looked fine after a week or two. The molecular methods we described in Chapter 4 changed that picture completely.

What a Single Course Actually Does

The landmark study on this question was published in 2018 by Albert Palleja and colleagues at the University of Copenhagen. The design was elegant and unsettling. Twelve healthy young men — average age twenty-four, no recent antibiotic use, no underlying illness — were given a four-day course of three broad-spectrum antibiotics: meropenem, gentamicin, and vancomycin. This combination was chosen to mimic the regimen a patient might receive for a serious hospital-acquired infection. The volunteers provided stool samples at baseline, at the end of treatment, and then at eight time points over the following six months. The samples were analysed by deep shotgun metagenomic sequencing, the technique that allows researchers to identify every microbial species present and estimate its abundance without the biases of culture [REF:palleja2018].

The headline result, repeated in news coverage at the time, was reassuring: most of the bacterial species present at baseline had returned to detectable levels by day forty-two. The microbiome was “resilient.” The word *resilient* did a lot of work in that coverage. What it obscured was everything in the paper that came after the headline.

First, “most” was not “all.” Nine common species that had been present in all twelve subjects at baseline were still undetectable in most subjects six months later. These were not obscure organisms. They included species of *Bifidobacterium adolescentis*, *Coprococcus eutactus*, and *Methanobrevibacter smithii* — species associated with short-chain fatty acid production, immune regulation, and hydrogen metabolism in the colon. The researchers' wording was cautious: these species had not recovered “during the observation period.” The implication, unstated but clear, was that they might not recover at all.

Second, the community that did come back was not the community that had left. Overall diversity — the number of species present, weighted by their relative abundances — remained below baseline at six months. More importantly, the *functional* composition of the community had shifted. Genes involved in virulence, in biofilm formation, and in antibiotic resistance were enriched in post-treatment samples. The bacteria that had survived the antibiotic onslaught and expanded into the vacated niches were, on average, more aggressive and less cooperative than the ones they had replaced. The forest had regrown, but it was a forest of weeds.

Third — and this was the finding that made microbiome researchers most uncomfortable — the post-treatment communities contained forty-seven new genes conferring resistance to a variety of antibiotics, including some that the volunteers had never received. These were not novel mutations. They were resistance genes that had been present all along, at low frequency, in minority members of the community. The antibiotic treatment had acted as a selective sieve, enriching for the carriers. When the community regrew, it regrew from a founder population that was already more antibiotic-resistant than the one it replaced.

One four-day course. Twelve healthy young men. A six-month shadow.

The Concentration Gradient That Nobody Prescribes

To understand why antibiotics do this much collateral damage, it helps to think about where the drug actually goes when you swallow it. When a patient takes an oral antibiotic, the drug is absorbed across the small intestinal wall into the bloodstream, from which it is distributed to the site of infection — a lung, a kidney, a surgical wound. But absorption is never complete, and excretion is never instantaneous. A substantial fraction of the dose passes through the small intestine unabsorbed and arrives in the large intestine, where the densest part of the microbiome lives. A further fraction, already absorbed, is secreted back into the gut via the bile — the so-called enterohepatic circulation. For some antibiotics, the concentration that reaches the colon is a small fraction of the blood level; for others, it is much higher than anywhere else in the body.

This means that the dense bacterial populations of the colon — *Bacteroides*, *Faecalibacterium*, *Roseburia*, *Bifidobacterium*, and the hundreds of less-abundant species that live alongside them — are exposed to antibiotic concentrations they did not need, in pursuit of a pathogen that is almost never in the colon. The “target” of the prescription is a streptococcus in the throat, a *Staphylococcus aureus* in a wound, an *E. coli* in the bladder. The *victims* of the prescription are the trillions of bystanders who happen to share the same body.

Pharmacologists have a phrase for this kind of exposure: *off-target effect*. It is the same phrase used when a cancer drug damages healthy tissue, or when a psychiatric drug disturbs sleep. But there is an important difference. When a chemotherapy drug damages the bone marrow, clinicians monitor the damage and stop if it becomes too severe. When an antibiotic damages the gut microbiome, nobody is looking. The prescription is written for the infection. The bystander community is neither monitored before nor counted after. In the vocabulary of clinical medicine, the microbiome is not yet an organ; it is collateral.

18.2 Short-Term Disruption and Long-Term Scars

The phrase “collateral damage” suggests something transient, the way a bruise is transient. And in the short term, that is how antibiotic disruption feels to most patients — a few days of loose stools, perhaps a bit of bloating, occasionally a yeast infection, and then back to normal. The “back to normal” part is an illusion, but it is a convincing one, because the host-level symptoms usually resolve even when the community underneath has not.

To see what the damage actually looks like in time, and how long it lasts, we need to follow three time-scales: hours, months, and years.

Hours and Days: The First Hit

Within twelve to twenty-four hours of the first oral dose of a broad-spectrum antibiotic, bacterial density in the colon begins to fall. By day three, in most adults, total faecal bacterial load has dropped by one to two orders of magnitude — that is, by a factor of ten to one hundred. Species diversity, measured by any of the standard ecological indices, drops sharply. The composition becomes *dominated* rather than *distributed*: a few surviving species, often organisms that were

present as minority members before treatment, expand into the empty niches and come to represent a large share of the total community. *Enterococcus* species are classic examples. So are certain *Enterobacteriaceae*, including the kinds of *E. coli* and *Klebsiella* that we would prefer not to see multiplying unchecked in the gut [REF:dethlefsen2011].

This early phase is also when patients notice the clinical side-effects. Antibiotic-associated diarrhoea affects somewhere between five and thirty per cent of patients, depending on the drug; for clindamycin and broad-spectrum beta-lactams, the rate is at the high end. Most cases are mild and self-limiting, caused by loss of the normal fermentative community that processes undigested carbohydrates in the colon. Without that community, carbohydrates reach the distal bowel intact, draw water osmotically, and produce diarrhoea. The same loss removes a protective function: the commensals that normally outcompete pathogens for nutrients and attachment sites are no longer there to do it.

The most severe version of this failure is *Clostridioides difficile* colitis — an infection that, almost uniquely among gut pathogens, is essentially a disease of antibiotic use. *C. difficile* spores are ingested from the environment all the time, but they cannot establish in a healthy, competitive gut community. When antibiotics remove the competition, the spores germinate, the bacterium colonises the damaged mucosa, and it releases toxins that cause severe inflammation and diarrhoea. In its worst form, *C. difficile* colitis produces life-threatening bowel perforation and requires emergency surgery. It kills approximately thirty thousand people per year in the United States alone, and almost every case is traceable to a prior course of antibiotics — often antibiotics prescribed for a trivial or questionable indication [REF:lessa2015]. The irony is stark: a drug prescribed to treat an infection sometimes causes one that is considerably worse.

Months: Partial Recovery

If the patient survives the acute phase — as the great majority do, with or without symptoms — what happens next is a long, gradual, partial return toward baseline. The Pallega study captured this phase in detail. Bacterial density recovers fairly quickly; within two to three weeks of the last dose, stool samples again look “full” under the microscope. Diversity recovers more slowly, usually taking one to two months. And composition — the precise identity and proportion of the species present — recovers most slowly of all, and often incompletely.

A different landmark paper, published by David Relman’s group at Stanford in 2011, followed three adults through two courses of ciprofloxacin, spaced six months apart. The first course reduced bacterial diversity substantially; by the time of the second course, the community had recovered — but not fully, and not to exactly the same configuration it had before. The second course produced a similar disruption, and the recovery from *that* course was less complete than the first. Each insult left a residue [REF:dethlefsen2011].

This raises an important point about how recovery should be measured. If we ask, “Did the bacterial community return to something that *looks* like a normal human microbiome?”, the answer is usually yes. The returning community has human-like numbers and human-like proportions of the dominant phyla. If we ask instead, “Did the community return to the specific configuration that existed in *this individual* before treatment?”, the answer is often no. The post-antibiotic community is a normal microbiome, but it is a *different* normal. It belongs to the individual’s post-treatment life, not to their pre-treatment life.

For a young, healthy adult with a single course of antibiotics for a real infection, this distinction may

be academic. The new microbiome is different, but probably functions well enough. For a child, or a chronically ill patient, or someone who has had many courses of antibiotics over a lifetime, the distinction matters a great deal.

Years: The Cumulative Shadow

The long-term consequences of antibiotic use — the ones that show up years or decades later — are the hardest to study and, not coincidentally, the ones Blaser has spent most of his career worrying about. The fundamental problem is that you cannot run a randomised controlled trial in which one group of children receives antibiotics and the other does not, and then follow both groups for thirty years to see who develops asthma, obesity, inflammatory bowel disease, or diabetes. The ethical and logistical barriers are insurmountable. What researchers can do, and have increasingly done, is look at very large observational cohorts — datasets containing millions of children, linked to national prescribing records and subsequent health outcomes — and ask whether the children who received antibiotics at particular ages went on to develop particular diseases more often than those who did not.

These studies cannot prove causation. They can only show association. An association between antibiotic exposure in the first year of life and a later diagnosis of asthma could, in principle, reflect a confounder: perhaps children who wheeze are more likely to be given antibiotics, and are also more likely to be diagnosed with asthma later. This kind of “reverse causation” is a real concern, and the best studies have gone to considerable lengths to address it — by excluding respiratory antibiotics from the analysis, by controlling for infection history, by comparing siblings who differ in their antibiotic exposure, and by applying causal inference techniques designed to strip out bias.

The associations that survive this scrutiny are uncomfortable. Large cohort studies have linked antibiotic exposure in the first two years of life to increased risks of childhood asthma, allergic rhinitis, atopic dermatitis, obesity, inflammatory bowel disease, juvenile idiopathic arthritis, and autism spectrum disorder — each association modest in magnitude, each of them repeatedly replicated in different populations [REF:aversa2021]. The effect sizes are not alarming for any one condition: a fifteen-to-thirty per cent increase in risk is typical, and a child who receives a single course of antibiotics for a genuine infection is not in a medical emergency. What is alarming is the cumulative picture, and the dose-response relationship. More courses produce more risk. Earlier exposure produces more risk than later. Broad-spectrum exposure produces more risk than narrow-spectrum. The pattern is exactly what you would expect if some fraction of the modern epidemic of childhood immune and metabolic disease were, in part, a consequence of ecosystem disruption during the developmental window when the microbiome is still being assembled.

This is the window we turn to next.

18.3 Early-Life Antibiotic Exposure and Lifetime Consequences

In Chapter 3, we described the assembly of the infant microbiome as a developmental process — a sequence of ecological successions that begins with the birth canal, continues through breastmilk, and settles into something resembling an adult community somewhere around the third birthday. This is one of the most consequential developmental processes in human biology. The microbes that establish themselves during the first thousand days educate the immune system, calibrate the

metabolism, and occupy the niches that will be hardest to reclaim later. Disrupt the process once, early enough and severely enough, and you may shape the rest of the life.

The Kenneth Ley Experiments

The strongest evidence for this claim comes from experiments that could only be done in animals. In 2012, a team led by Ilseung Cho and Martin Blaser at NYU reported a series of experiments on young mice given subtherapeutic doses of antibiotics — the kind of low-dose, continuous exposure used for decades in the livestock industry to promote growth. The mice were given penicillin, vancomycin, chlortetracycline, or a combination, in doses designed to mimic agricultural exposure rather than to treat any specific infection. The intervention began at weaning and continued for a few weeks [REF:cho2012].

The mice gained weight. More precisely, they developed altered body composition: more fat, different fat distribution, changes in hepatic lipid metabolism, altered expression of genes involved in lipid handling. The antibiotic-exposed animals were not sick — they looked like normal, healthy mice who happened to be slightly fatter than expected. The microbiome changes that accompanied these metabolic changes were modest in taxonomic terms but functionally significant: shifts in the proportions of the dominant phyla, changes in genes involved in carbohydrate metabolism and short-chain fatty acid production.

A follow-up study, published two years later, went further. The team showed that giving the antibiotic exposure *only* during the early-life window — the first few weeks after weaning — produced lasting metabolic changes, even though the microbiome eventually recovered its pre-antibiotic composition [REF:cox2014]. The gut community healed. The body did not. Something about the community's state during the early-life window had programmed the animal's later physiology, and once the window closed, the programming was set. You could restore the microbiome and still have the altered animal.

Transplant experiments confirmed the causal direction. When the researchers transplanted microbiota from antibiotic-exposed young mice into germ-free recipients, the recipients developed the same metabolic changes. When they transplanted microbiota from control mice, the recipients did not. The altered community was not just a marker of altered physiology; it was the cause of it. And the critical window — the period during which the alteration had to happen in order to produce the lasting effect — was early, brief, and easily missed.

The Human Parallel

Extrapolating from mice to humans is a dangerous business, and nowhere more dangerous than in microbiome research, where species differences in gut community structure, immune development, and metabolism are substantial. But the mouse experiments were designed to be informative about the human question, and their results lined up suspiciously well with what observational studies of children had been suggesting for years. Children given antibiotics before age two are, on average, modestly heavier than their peers later in childhood. Children given multiple courses of broad-spectrum antibiotics in the first year of life are at increased risk of obesity, metabolic disturbance, and immune disorders, as noted above. The human evidence is correlational, but the mouse evidence is experimental, and the two converge on the same conclusion: the developing microbiome is vulnerable in a way the adult microbiome is not, and the vulnerability has lifelong consequences.

One detail deserves emphasis. The agricultural practice that the mouse experiments modelled —

feeding low-dose antibiotics continuously to young livestock — was not adopted because someone had a theory about microbiomes. It was adopted empirically, in the 1950s, when farmers noticed that young chickens and pigs given antibiotic-laced feed grew faster and produced more meat per unit of feed. The practice spread worldwide and was, for decades, one of the main uses of antibiotics in volume terms: more antibiotic by weight went into livestock feed than into human medicine. We have, in other words, accidental empirical evidence from billions of animals that early-life, low-dose antibiotic exposure alters growth. Blaser’s contribution was to point out the uncomfortable implication: if it does it in farm animals, there is no obvious reason it would not do it in children.

The Caesarean Complication

A full account of early-life microbiome disruption must also mention a procedure that is not usually thought of as an antibiotic exposure but effectively always includes one: the elective caesarean section. In most high-income countries, caesarean delivery is performed with prophylactic antibiotics given to the mother shortly before the skin incision, to reduce the risk of post-surgical wound infection. These antibiotics cross the placenta and reach the baby before the umbilical cord is cut. The first substance the newborn encounters is not the vaginal microbiome, but ampicillin or a cephalosporin.

We described the consequences of bypassing the birth-canal microbial transfer in Chapter 3: caesarean babies acquire a starter community dominated by skin and environmental organisms rather than the maternal vaginal and intestinal flora that vaginally-born babies inherit. Add to this the additional disruption of intrapartum antibiotic exposure, and the result is a newborn whose first microbiome is both incomplete and partially sterilised. The epidemiological signal is consistent with this mechanism: caesarean-born children show modestly elevated risks for many of the same conditions that are associated with early-life antibiotic use — asthma, allergy, obesity, immune disorders [REF:chu2017].

We do not yet know how to fully correct for this disruption. Experiments with “vaginal seeding” — swabbing the newborn with maternal vaginal fluid after caesarean delivery — have shown partial restoration of the expected microbial profile, but the clinical effects on later health outcomes are still being evaluated [REF:dominguezbello2016]. The point is not that caesarean sections should be avoided — they save lives, and in many cases are the only safe option for mother or baby. The point is that modern delivery practice has routinely imposed a double microbial insult on newborns without appreciating that either one was happening.

18.4 Antibiotic Resistance Genes in the Commensal Microbiome — A Hidden Reservoir

Most of what the public hears about antibiotic resistance concerns pathogens: MRSA in hospitals, resistant *E. coli* in urinary tract infections, multi-drug-resistant tuberculosis. The implicit mental model is that “resistance” is a property of “bad” bacteria — the ones that cause disease — and that the solution is to prescribe antibiotics more cautiously, develop new drugs, and contain the resistant pathogens in hospitals. This model is not wrong, exactly, but it is radically incomplete. It misses the fact that the largest reservoir of antibiotic resistance genes in most human bodies is not in the pathogens but in the commensals.

The Resistome

Microbiome researchers use the term *resistome* to describe the complete collection of antibiotic resistance genes in a community — all of them, whether in pathogenic or commensal bacteria, whether currently active or dormant, whether on chromosomes or on mobile genetic elements that can be transferred between cells. The resistome is, in some sense, a geological record of the community's antibiotic history: every gene in it is there because, at some point in the evolutionary past, an ancestor of the current community acquired it and found it useful enough to keep.

The surprise, when researchers first began characterising human gut resistomes systematically, was how large they were. Even in individuals with no recent antibiotic exposure, the gut microbiome carries hundreds to thousands of resistance genes, distributed across dozens of bacterial species [REF:hu2013]. Resistance to tetracyclines and beta-lactams is near-universal. Resistance to macrolides, aminoglycosides, and fluoroquinolones is common. Some of these genes are very old — older than the antibiotics we now use to treat human infections. The first antibiotic resistance genes evolved in the soil, where antibiotic-producing organisms have been making antibiotics, and competing organisms have been evolving resistance to them, for hundreds of millions of years. When humans began extracting antibiotics from soil organisms and using them as medicine, we imported, along with the drugs, the resistance infrastructure that had evolved alongside them.

What antibiotic treatment does to the resistome is not to *create* resistance genes — they are already there — but to *enrich* them. The sensitive bacteria die; the resistant bacteria survive and multiply. Genes that were present in one per cent of the community can be in thirty per cent a week later. And because many resistance genes sit on mobile genetic elements — plasmids, integrons, transposons, and the prophages we discussed in Chapter 16 — they can jump from one bacterial species to another. A resistance gene that was quietly present in a commensal *Bacteroides* can end up, after a few rounds of conjugation, in an opportunistic *E. coli* or a nascent *C. difficile* strain.

The Pool Contamination Problem

This dynamic produces an uncomfortable implication. Every course of antibiotics a patient takes not only treats the target infection but also updates the patient's personal resistome. The update is usually modest, but it is cumulative, and the gene pool of resistance genes is shared — through the family, through the workplace, through the healthcare system — with everyone the patient contacts. Antibiotic resistance, in this view, is not just a property of individual pathogens; it is a property of human populations.

A 2019 study by Yassour and colleagues followed Finnish children from birth to age three, sampling their gut microbiomes monthly and tracking their antibiotic exposures. The researchers found that resistance genes accumulated in the children's microbiomes over time, with each course of antibiotics producing a detectable uptick. Even more striking, some of the resistance genes detected in the children were genes for antibiotics they had never received — genes that had presumably been acquired by commensals through horizontal transfer, carried along as passengers, and then selected for when any antibiotic was given [REF:yassour2016]. The children were accumulating a resistance portfolio. They were not the patients for whom these resistance genes would matter, but they were the carriers who would pass them on.

This is the hidden cost of antibiotic use that traditional resistance metrics miss. When a hospital reports its rates of MRSA or VRE, it is counting clinically apparent resistant infections. It is not counting the slow accumulation of resistance determinants in the gut flora of every patient, every

visitor, every healthcare worker, and every patient's family at home. The hospital's resistance ledger looks manageable. The population's looks considerably less so.

18.5 *Candida* Blooms and the Kingdom That Fills the Vacuum

There is a group of organisms in the gut that we have discussed at length in this volume but have not yet mentioned in this chapter: the fungi. The oversight is appropriate, because for most healthy people, the gut mycobiome is a minor, well-regulated component of the microbial community — a minority by numbers, a minority by metabolic contribution, and normally kept in check by the bacterial majority. Antibiotics change that.

We touched on this phenomenon in Chapter 10, when we described how *Candida albicans* — the most common commensal yeast in the human gut — undergoes dramatic expansion after antibiotic treatment. The mechanism is straightforward: bacteria and fungi compete for the same resources — nutrients, attachment sites, and growth factors — and in a healthy gut, the bacteria usually win, because they are more numerous, they grow faster, and they produce metabolic by-products that suppress fungal proliferation. Remove the bacterial majority with a broad-spectrum antibiotic, and the fungi, which are intrinsically resistant to antibacterial drugs, find themselves in a newly empty environment with abundant nutrients and no competition. *Candida* populations in stool samples can increase by two or three orders of magnitude within a week of starting antibiotic treatment [REF:dollive2013].

For most patients, this fungal bloom is asymptomatic or produces only mild symptoms — oral thrush, vaginal candidiasis, diaper rash in infants. These are the visible consequences of an invisible shift in the gut ecosystem. For immunocompromised patients, the consequences can be much more serious, including invasive candidiasis and bloodstream infection. But the more interesting question, for the purposes of this chapter, is not what happens to the patient during the *Candida* bloom. It is what happens to the ecosystem, and what happens when the patient stops taking the antibiotic.

This is where the interkingdom dynamics we described in Chapter 10 come back into play. *Candida* is not a passive opportunist. Its expansion in the post-antibiotic gut actively remodels the environment in ways that make bacterial recolonisation harder. Fungal hyphae provide physical surfaces that can be colonised by opportunistic bacteria, creating mixed biofilms. *Candida* metabolism acidifies the local environment and produces signalling molecules that influence bacterial gene expression. Most importantly, *Candida* primes the host immune system in ways that favour inflammation — a pattern we discussed in Chapter 10 in the context of inflammatory bowel disease, where gut fungal expansion is a consistent feature. When the antibiotic is stopped and the bacterial community attempts to regrow, it must do so in an environment that has been reshaped by the intervening fungal bloom.

The clinical implications of this are still being worked out. It is clear, from observational studies, that patients with persistent gut *Candida* expansion after antibiotic treatment have worse microbiome recovery and higher rates of subsequent bacterial infections, including *C. difficile* [REF:markey2018]. It is also clear that the question of what to do about it is unresolved. Antifungal drugs such as fluconazole suppress *Candida* but introduce their own ecological disruption to the fungal community. *Saccharomyces boulardii*, a probiotic yeast we will return to in the probiotics chapter, has been used empirically to displace *Candida* and promote microbiome recovery, with

mixed but generally encouraging results. The idea that one fungus might be used as a defence against another's expansion is ecological medicine in its most literal sense.

For now, the key point is that the “kingdom blind spot” we discussed in Chapter 10 is not just a research problem. It is a clinical one. A clinician who prescribes antibiotics is implicitly prescribing a fungal expansion. The prescription note mentions neither.

18.6 The Recovery Phase — What Actually Happens When Antibiotics Stop

Imagine you could look into the gut of a patient who has just finished a course of antibiotics, with a time-lapse camera capable of following bacteria day by day. What you would see, over the following weeks, is one of the great dramas of ecological biology: a recolonisation race, played out in real time, with the outcome depending largely on who gets there first.

The Vulnerability Window

In the first week after the last dose, the gut community is at its minimum. Total bacterial density is low. Diversity is collapsed. The survivors — organisms that happened to be antibiotic-resistant, or that occupied sanctuary niches where the drug did not reach, or that existed as spores or other dormant forms — are scattered through an environment that is, from their perspective, unusually empty and unusually resource-rich. The normal competitive pressures that constrain any individual species in a healthy microbiome have been temporarily lifted.

This is the *vulnerability window*, and it lasts, depending on the antibiotic and the individual, somewhere between one and four weeks. During this time, the gut is abnormally permissive. Species that would normally struggle to establish, because they cannot compete with the incumbent community, can now find empty niches. Species that enter the gut in small numbers — from food, from the environment, from another person's skin — have a better chance of taking hold than they would in a healthy gut. The race is on.

The race has several competitors. There are the survivors of the antibiotic insult, regrowing from whatever remnant populations they managed to preserve. There are the organisms flowing in from upstream — from the mouth, from food, from the environment. There are the organisms in the healthcare environment, if the patient is in hospital, including some that are notorious for exploiting exactly this window: *Clostridioides difficile*, vancomycin-resistant *Enterococcus*, carbapenem-resistant *Enterobacteriaceae*. And, in a growing number of clinical studies, there are the organisms deliberately introduced by the clinician: probiotics, fermented foods, or — in the most radical version — transplanted faecal communities from a healthy donor.

What determines the outcome? Ecologically, the answer is: *who arrives first*, and in *what numbers*. This is the principle of *priority effects* in community ecology. When a habitat has been cleared and is being recolonised, early arrivers have a disproportionate influence on the final community structure, because they establish the conditions under which later arrivers must compete. A niche filled early by one species is not available to another. A metabolite produced early by one organism shapes the environment for everything that follows. The species that win the recolonisation race are not necessarily the ones that would dominate a mature, equilibrium community; they are the ones that happen to be abundant during the first days of the vulnerable window.

This insight has substantial therapeutic implications, which we will return to in the probiotics

chapter and in our discussion of faecal microbiota transplantation. For now, the point is that the recovery phase is not a passive process of the old community reassembling itself. It is an active, competitive, ecologically contingent process in which the starting conditions matter enormously. The “natural” recovery that happens when a patient stops their antibiotics and does nothing else is one possible outcome. It is not the only one.

Why Recovery Is So Often Incomplete

Return now to the Palleja study. Why did those nine bacterial species fail to recover at six months? Four possibilities, none of them reassuring, can be read from the data.

First, some species may have been *locally extinct*. If the antibiotic killed every cell of a given species in the gut, that species can only return if it re-enters from outside — from food, from contact with another person, from the environment. In an ancestral human living in close contact with dirt, family members, and livestock, re-entry would be relatively easy. In a modern adult living in a clean apartment and eating processed food, it may be nearly impossible. Extinction at the individual level becomes effectively permanent.

Second, some species may have depended on cooperative interactions with other species that were themselves eliminated. A bacterium that consumes the metabolic by-products of another bacterium cannot thrive if its partner is gone. A species that requires a specific vitamin produced by another species has the same problem. The gut microbiome contains extensive networks of such metabolic dependencies, and disrupting several species at once can cause cascading losses that are hard to reverse even after the antibiotic is withdrawn.

Third, some species may have been displaced by successor communities that now occupy the niches they used to hold. Once another organism has settled into a niche and established itself, the original occupant faces a competitive disadvantage in trying to return. The ecological door has closed.

And fourth, some species may still be present at very low, undetectable levels and may yet recover on a time-scale longer than the study measured. The six-month cut-off of the Palleja paper was dictated by practical constraints, not biological ones. We do not yet know the true time-scale of complete recovery — if it happens at all.

These four mechanisms are not mutually exclusive. They act together, and they act differently in different individuals, which is part of why recovery trajectories after antibiotic treatment are so variable. Some people seem to bounce back to essentially the same community they started with. Others do not. The factors that distinguish these groups — age, baseline diversity, diet, environmental exposure, genetics, and the specific antibiotic regimen — are still being untangled.

This is the background against which Chapter 19 opened with Sarah’s story. When Sarah received her third antibiotic course for recurrent urinary tract infection, her gut had not fully recovered from the second, and the community that regrew was progressively less able to provide the colonisation resistance that would normally keep opportunistic *E. coli* from proliferating. Each course had worked clinically, in the short term. Each course had left her ecosystem in a more vulnerable state than the one before. By the time the clinicians had run out of empiric options, Sarah’s problem was no longer an infection. It was an ecosystem that had been blunted by the very therapy meant to help it, and was now unable to defend itself.

18.7 Towards Precision: Narrow-Spectrum and Microbiome-Sparing Approaches

If the argument of this chapter is correct — that antibiotics, particularly broad-spectrum antibiotics, cause substantial collateral damage to the commensal microbiome, and that this damage has real consequences for patients — then it follows that the correct response is not to abandon antibiotics, but to use them better. The word “better” in this context means something more specific than “prescribing less.” It means matching the therapy more precisely to the target, so that the off-target damage is minimised.

This is not a new idea. The physicians who first used antibiotics in the 1940s understood the principle perfectly well: treat the specific bacterium causing the specific infection, with the most targeted drug available. What changed over the following decades was not the principle but the practice. As diagnostic laboratories became slower relative to clinical urgency, as culture-and-sensitivity results began to arrive too late to guide the initial prescription, and as broad-spectrum drugs became available that made the question of “which organism is it?” seem almost obsolete, empiric broad-spectrum therapy became the default. It was faster, easier, and “safer” in the sense that it was less likely to miss the pathogen. The ecological cost of that safety was invisible, so it was ignored.

Reversing this drift requires progress on several fronts.

Faster Diagnostics

The first front is diagnostic speed. The traditional workflow for bacterial identification — culture the specimen, wait one to three days for growth, test antibiotic susceptibility, wait another day for results — is too slow to guide the initial prescription in most acute infections. The physician has to start treatment before the laboratory can say what organism is present, and by the time the results are back, the empiric therapy is usually either working (in which case nobody bothers to change it) or not working (in which case switching is complicated by the partial treatment already received).

This workflow is finally changing. Rapid molecular diagnostics — PCR-based tests, mass spectrometry (MALDI-TOF), and metagenomic sequencing — can now identify bacterial pathogens from a clinical specimen in hours rather than days, and the same technologies can increasingly detect resistance genes directly, without waiting for the bacteria to grow. In some settings, a urine sample can be analysed for pathogen identity and antibiotic susceptibility in under an hour. In others, a bloodstream infection can be characterised overnight rather than over days [REF:goswami2023]. These advances have not yet reached most primary care settings, where the great majority of antibiotic prescriptions are written, but they are reaching there — slowly, patchily, and driven more by cost considerations than by microbiome concerns. The ecological consequences of faster diagnostics have been a pleasant side-effect rather than a primary motivation, but they are real. A prescription written against a known organism can use a narrower drug, administered for a shorter duration, than a prescription written against a statistical guess.

Narrow-Spectrum Drugs

The second front is the revival of interest in narrow-spectrum antibiotics — drugs that target specific bacterial families rather than the broad sweep that current broad-spectrum agents cover. For much of the antibiotic era, narrow-spectrum agents were considered commercially unattractive. A drug that works only against *Streptococcus pyogenes* has a smaller market than one that works

against streptococci, staphylococci, and a range of gram-negative bacilli. Pharmaceutical companies developing new antibiotics naturally preferred broader-spectrum candidates. The result was a pipeline biased toward exactly the kind of drugs that do the most collateral damage.

This bias is now being reconsidered, partly because broad-spectrum drugs are increasingly being held in reserve as last-line therapies in the face of resistance, and partly because microbiome research has made the collateral damage concrete. A narrow-spectrum antibiotic that leaves the bulk of the commensal community intact is now, for the first time, being valued for what it does *not* do. Drugs such as fidaxomicin — a narrow-spectrum macrocyclic antibiotic that targets *Clostridioides difficile* with minimal effect on the wider gut community — represent the kind of microbiome-sparing agent that was previously considered a curiosity and is now considered a template [REF:louie2011].

The logic of narrow-spectrum therapy is, however, only as good as the diagnostic precision that makes it possible. If you do not know what organism you are treating, a narrow-spectrum drug is a liability: it might miss. The diagnostic and pharmacological fronts advance together or not at all.

Shorter Courses

The third front is duration. For most infections, the duration of antibiotic therapy was set decades ago by expert opinion rather than by controlled trials. Ten days of penicillin for a streptococcal sore throat. Fourteen days of treatment for a urinary tract infection. Twenty-one days for a complicated pyelonephritis. These numbers were chosen to be safely long enough that the infection would be eradicated in everyone, including the slow responders. They were not chosen to minimise collateral damage, because at the time no one was thinking about that.

A growing body of modern trial evidence shows that shorter courses — sometimes *much* shorter courses — achieve the same clinical cures with less collateral damage. Three days of antibiotics for uncomplicated cystitis is now standard in many guidelines, replacing the traditional seven-to-ten-day course. Five days of antibiotics for community-acquired pneumonia produces outcomes indistinguishable from ten days. Seven days of treatment for bacteraemia is, in carefully selected patients, as effective as fourteen [REF:spellberg2016]. The pattern is consistent: the long courses that were standard for decades were longer than necessary, and the shortening delivers benefits both to the individual patient (fewer side-effects, less disruption) and to the community (less resistance selection). The guideline updates are happening slowly, because changing clinical practice is always slow, but the direction of travel is clear.

Protecting the Bystanders

The fourth front is the most speculative, but perhaps the most interesting. It asks: is there a way to protect the commensal microbiome *during* antibiotic therapy, so that the target infection is treated while the bystander community is shielded from collateral damage? The answers coming from research laboratories are beginning to include ideas that would have sounded absurd a decade ago.

One approach is to deliver antibiotics in a form that keeps them out of the colon. Oral beta-lactamases, taken alongside oral antibiotics, can degrade any drug residue that reaches the lower gut before it can do damage to the commensal community. A drug called ribaxamase was developed specifically for this purpose — to preserve the colonic microbiome during intravenous beta-lactam therapy — and early trials have shown that it substantially reduces the disruption associated with such treatment, including a reduction in *C. difficile* infection rates [REF:kokai2019]. The approach

is still experimental, but the principle — *selective decontamination of the contamination* — is novel and promising.

Another approach is to use bacteriophages, which brings us back to where this chapter began and forward to where the next chapter goes. Phages, as we have seen, are exquisitely specific. A phage that targets a particular strain of *E. coli* does not touch the rest of the community. In principle, phage therapy is the ultimate narrow-spectrum antibacterial treatment: it is a treatment for one strain of one species, and nothing else. The ecological consequences for the commensal community are, by design, minimal. We will explore what this means clinically in Chapter 19.

And a third approach, at the frontier of what is currently possible, is to combine antibiotic therapy with deliberate microbial reconstitution — either by giving probiotics during the antibiotic course to maintain a defensive population, or by giving faecal microbiota transplants afterwards to repopulate the damaged community. These are ideas we will develop in later chapters of this volume. For now, the point is that the mental model is shifting. For seventy years, antibiotic therapy was conceptualised as a duel between the drug and the pathogen, with the microbiome as inert background. That model is now being replaced by one in which the microbiome is an active, consequential participant — an ecosystem whose protection is part of the treatment plan rather than an afterthought.

A Different Kind of Stewardship

The standard response to the antibiotic crisis has been “antibiotic stewardship” — the set of institutional programmes designed to ensure that antibiotics are prescribed only when necessary, at the right dose, for the right duration, with the right drug. Stewardship is good. It is also, as currently practised, almost entirely concerned with preventing the emergence of resistant pathogens. The ecological damage to the commensal microbiome — the nine species that do not come back, the resistance genes that accumulate in healthy children’s guts, the metabolic shifts that persist for years — is not part of most stewardship programmes. It is not measured, not reported, and therefore not optimised against.

A broader form of stewardship is beginning to emerge. It treats the commensal microbiome as a patient-level asset worth protecting, measures antibiotic exposure in terms of ecological cost as well as pharmacological effect, and values narrow-spectrum, short-duration, microbiome-sparing therapy even when the broader-spectrum alternative would be clinically adequate. The call is not for less antibiotic prescribing but for *better* antibiotic prescribing: prescribing that accounts for the full cost of the treatment, including the cost to the organ that has, so far, been asked to bear that cost in silence.

Seventy-five years after Fleming’s Nobel Lecture, we are beginning to understand the dimensions of the warning he issued. It was not only that bacteria would evolve resistance to the drugs we use against them, although they have. It was also that those drugs, used the way they came to be used, would reshape the ecosystems we carry inside us — silently, cumulatively, and for keeps. The forest analogy is not an exaggeration. The cost, counted properly, is not negligible. And the question — what is a better way? — takes us directly into the next chapter, where we meet the oldest answer to that question, revived just in time.

Key References — Chapter 18

- [REF:fleming1945] Fleming A (1945). Penicillin. Nobel Lecture, 11 December 1945. Nobel Foundation, Stockholm.
- [REF:blaser2014] Blaser MJ (2014). *Missing Microbes: How the Overuse of Antibiotics Is Fueling Our Modern Plagues*. Henry Holt and Company, New York.
- [REF:palleja2018] Palleja A, Mikkelsen KH, Forslund SK et al. (2018). Recovery of gut microbiota of healthy adults following antibiotic exposure. *Nat. Microbiol.*, 3(11), 1255–1265. doi:10.1038/s41564-018-0257-9
- [REF:dethlefsen2011] Dethlefsen L & Relman DA (2011). Incomplete recovery and individualized responses of the human distal gut microbiota to repeated antibiotic perturbation. *Proc. Natl. Acad. Sci. USA*, 108(Suppl 1), 4554–4561. doi:10.1073/pnas.1000087107
- [REF:lessa2015] Lessa FC, Mu Y, Bamberg WM et al. (2015). Burden of *Clostridium difficile* infection in the United States. *N. Engl. J. Med.*, 372(9), 825–834. doi:10.1056/NEJMoa1408913
- [REF:aversa2021] Aversa Z, Atkinson EJ, Schafer MJ et al. (2021). Association of infant antibiotic exposure with childhood health outcomes. *Mayo Clin. Proc.*, 96(1), 66–77. doi:10.1016/j.mayocp.2020.07.019
- [REF:cho2012] Cho I, Yamanishi S, Cox L et al. (2012). Antibiotics in early life alter the murine colonic microbiome and adiposity. *Nature*, 488(7413), 621–626. doi:10.1038/nature11400
- [REF:cox2014] Cox LM, Yamanishi S, Sohn J et al. (2014). Altering the intestinal microbiota during a critical developmental window has lasting metabolic consequences. *Cell*, 158(4), 705–721. doi:10.1016/j.cell.2014.05.052
- [REF:chu2017] Chu DM, Ma J, Prince AL et al. (2017). Maturation of the infant microbiome community structure and function across multiple body sites and in relation to mode of delivery. *Nat. Med.*, 23(3), 314–326. doi:10.1038/nm.4272
- [REF:dominguezbello2016] Dominguez-Bello MG, De Jesus-Laboy KM, Shen N et al. (2016). Partial restoration of the microbiota of cesarean-born infants via vaginal microbial transfer. *Nat. Med.*, 22(3), 250–253. doi:10.1038/nm.4039
- [REF:hu2013] Hu Y, Yang X, Qin J et al. (2013). Metagenome-wide analysis of antibiotic resistance genes in a large cohort of human gut microbiota. *Nat. Commun.*, 4, 2151. doi:10.1038/ncomms3151
- [REF:yassour2016] Yassour M, Vatanen T, Siljander H et al. (2016). Natural history of the infant gut microbiome and impact of antibiotic treatment on bacterial strain diversity and stability. *Sci. Transl. Med.*, 8(343), 343ra81. doi:10.1126/scitranslmed.aad0917
- [REF:dollive2013] Dollive S, Chen YY, Grunberg S et al. (2013). Fungi of the murine gut: episodic variation and proliferation during antibiotic treatment. *PLoS ONE*, 8(8), e71806. doi:10.1371/journal.pone.0071806
- [REF:markey2018] Markey L, Shaban L, Green ER et al. (2018). Pre-colonization with the commensal fungus *Candida albicans* reduces murine susceptibility to *Clostridium difficile* infection. *Gut Microbes*, 9(6), 497–509. doi:10.1080/19490976.2018.1465158
- [REF:goswami2023] Goswami C, Fox S, Holden MTG & Connor TR (2023). Genomic approaches to rapid diagnosis of antimicrobial resistance. *Clin. Microbiol. Infect.*, 29(7), 841–847. doi:10.1016/j.cmi.2023.02.024
- [REF:louie2011] Louie TJ, Miller MA, Mullane KM et al. (2011). Fidaxomicin versus vancomycin for *Clostridium difficile* infection. *N. Engl. J. Med.*, 364(5), 422–431. doi:10.1056/NEJMoa0910812
- [REF:spellberg2016] Spellberg B (2016). The new antibiotic mantra — “shorter is better”.

JAMA Intern. Med., 176(9), 1254–1255. doi:10.1001/jamainternmed.2016.3646

- [REF:kokai2019] Kokai-Kun JF, Roberts T, Coughlin O et al. (2019). Use of ribaxamase (SYN-004), a beta-lactamase, to prevent *Clostridium difficile* infection in beta-lactam-treated patients: a double-blind, phase 2b, randomised placebo-controlled trial. *Lancet Infect. Dis.*, 19(5), 487–496. doi:10.1016/S1473-3099(18)30731-X

Chapter 19: Phage Therapy — The Comeback

19.1 A History: D'Hérelle, Soviet Medicine, and the Road Not Taken

In 1919, two years after he named the bacteriophage and published his discovery in the *Comptes Rendus* of the French Academy of Sciences, Félix d'Hérelle did something that no modern drug regulator would countenance. He drank a phage preparation himself, waited to see if anything happened, then gave it to his family members. When nobody fell ill, he administered phages to a twelve-year-old boy dying of severe dysentery at the Hôpital des Enfants-Malades in Paris. The boy recovered. D'Hérelle published the result and moved on to treating cholera patients in India, plague victims in Egypt, and dysentery outbreaks wherever he could find them. By the early 1920s, he was the most controversial microbiologist in the world — a self-taught outsider making extraordinary claims about a therapy that the scientific establishment could not decide was real [REF:dherelle1917].

We told part of this story in Chapter 16, when we described the discovery of phages and the bitter dispute between d'Hérelle and Jules Bordet over whether phages were truly living viruses or merely bacterial enzymes. But the story of phage *therapy* — the deliberate use of phages to treat human infections — has a trajectory of its own, and it is a trajectory shaped as much by geopolitics as by science. It is a story of a promising therapy that flourished in the East while the West forgot it existed, and of the extraordinary circumstances that are now bringing it back.

The Georgian Connection

The pivotal figure in the survival of phage therapy was a Georgian microbiologist named George Eliava. Born in 1892, Eliava trained in bacteriology in Tbilisi and Odessa before travelling to Paris in the early 1920s, where he met d'Hérelle at the Pasteur Institute. The two became collaborators and friends. Eliava was captivated by phage biology and by d'Hérelle's vision of therapeutic application. When he returned to Tbilisi, he carried that vision with him, and in 1923 he established a bacteriological research institute dedicated to studying and producing phages for clinical use [REF:kutateladze2010].

D'Hérelle visited Tbilisi twice, in 1933 and 1934, and spent approximately eighteen months working alongside Eliava — his longest sustained collaboration. Stalin himself had endorsed the project, recognising the strategic value of a therapy that could treat battlefield infections. The Tbilisi institute became the world's first purpose-built centre for phage research and production, and the two men began building a phage library: a collection of bacteriophages active against the most common human pathogens [REF:kutateladze2010].

The collaboration ended with a knock on the door. In 1937, during Stalin's Great Terror, George Eliava was arrested by the NKVD — Lavrenty Beria's secret police — and executed as an “enemy of the people.” The exact charge remains disputed; some accounts suggest political disfavour, others that Beria held a personal grudge. All of Eliava's papers and photographs were destroyed. He became a non-person, virtually unknown outside Georgia for forty years. D'Hérelle, devastated, returned to France and never went back to the Soviet Union [REF:kutateladze2010].

But the institute survived. Eliava's colleagues continued the work — quietly, under conditions of political repression, with whatever resources they could secure. Throughout the Soviet era, the Tbilisi institute (officially renamed the George Eliava Institute of Bacteriophage, Microbiology and Virology in 1988) produced phage preparations on an industrial scale. Soviet soldiers received phage cocktails as routine prophylaxis. Phage preparations were available in Georgian pharmacies without a prescription — as commonplace as aspirin. The science was real, the clinical use was widespread, and the results, while inconsistently documented by Western standards, were often impressive [REF:abedon2011].

Why the West Walked Away

Meanwhile, the West abandoned phage therapy almost entirely. The reasons were partly scientific and partly historical.

The scientific problems were genuine. Early phage preparations were often crude — poorly purified, inconsistently potent, and contaminated with bacterial debris that could cause fevers and inflammatory reactions. Clinical trials in the 1920s and 1930s produced mixed results, and the rigour of those trials, by modern standards, was poor. Some researchers used phages against infections that we now know were caused by organisms the phages could not infect. Others failed to account for the extreme specificity of phages — a preparation active against one strain of *Staphylococcus aureus* might be useless against the strain infecting a particular patient. Without proper strain matching, phage therapy was, in effect, a lottery.

But the decisive blow was not scientific. It was pharmacological. In 1928, Alexander Fleming discovered penicillin. By the early 1940s, industrial production of penicillin had begun, and the results were transformative. Penicillin worked against a broad range of bacteria. It did not require strain matching. It did not need to be individually prepared for each patient. It could be manufactured in vast quantities, shipped anywhere, stored indefinitely, and prescribed by any physician. It was, in every practical sense, the opposite of phage therapy: a universal solution where phages offered only a bespoke one.

The pharmaceutical industry chose antibiotics, and the funding followed. By the 1950s, phage therapy had virtually disappeared from Western medical literature. It persisted only in the Soviet Union and its satellite states — in Tbilisi, in Warsaw, in Wrocław — where it continued to be used clinically, studied scientifically, and refined practically, largely invisible to the English-speaking world.

The Cold War made this invisibility worse. Soviet-era publications were in Russian or Georgian, rarely translated, and published in journals that Western libraries did not carry. The few Western scientists who visited Tbilisi found an institute that was clinically productive but, after the Soviet collapse in 1991, physically crumbling. During the Georgian Civil War of the early 1990s, researchers reportedly stored phage cultures in their own homes — in personal refrigerators — to prevent the library from being lost. The institute survived, but just barely [REF:kutateladze2010].

The Return

The resurgence of phage therapy in the West was driven by a crisis that d'Hérelle could not have anticipated: the rise of antibiotic resistance. By the 2000s, the miracle drugs that had displaced phages were failing. Multi-drug-resistant bacteria — MRSA, carbapenem-resistant *Enterobacteriaceae*, extensively drug-resistant *Mycobacterium tuberculosis* — were causing infections that no available antibiotic could treat. The pipeline of new antibiotics had slowed to a trickle, because the

economics of antibiotic development are punishing: a drug that works is used for short courses and then held in reserve, producing far less revenue than a chronic-disease medication taken for years.

In this context, Western medicine began to look, with increasing urgency, at the therapy it had abandoned seventy years earlier. And when it looked, it found the Eliava Institute still standing — still producing phages, still treating patients, still maintaining a library of over six hundred characterised bacteriophages active against the most clinically important human pathogens [REF:zaldastanishvili2021].

19.2 Two Patients, Two Infections, Two Worlds

To understand what phage therapy actually looks like — not as a concept but as a clinical experience — it helps to follow two hypothetical patients through the same infection. Both have the same problem: a chronic, antibiotic-resistant urinary tract infection caused by multi-drug-resistant *Escherichia coli*, an infection that has recurred despite three courses of antibiotics over the past eighteen months. One patient follows the conventional Western pathway. The other travels to Tbilisi.

These are composite patients — not real individuals, but their stories are assembled from published case reports and documented clinical workflows at the Eliava Phage Therapy Center [REF:zaldastanishvili2021] [REF:leitner2021].

The Antibiotic Pathway: Sarah's Story

Sarah is fifty-two, a schoolteacher in Melbourne, and she has been battling recurrent UTIs for two years. The first infection responded to trimethoprim. The second did not — her GP switched to nitrofurantoin, which cleared the symptoms for three months before they returned. The third course was a fluoroquinolone, ciprofloxacin, which worked briefly but left Sarah with tendon pain and persistent gastrointestinal disturbance. Her urine culture now grows *E. coli* resistant to all three classes.

Sarah's experience is not unusual. Among patients with recurrent UTIs, resistance accumulates with each treatment cycle. A large cohort study of nearly 149,000 UTI patients found that by the sixth infection, sixty-five per cent of causative organisms were resistant to at least one antibiotic class, and twenty per cent were resistant to three or more [REF:dickstein2023]. Each course of empiric therapy — prescribed before culture results are available, targeting the most likely pathogen with the most likely drug — is a gamble. When the gamble fails, the patient has gained nothing except collateral damage to her microbiome.

And the collateral damage is real. A landmark 2018 study by Palleja and colleagues followed twelve healthy young men given a four-day course of three broad-spectrum antibiotics. Using deep metagenomic sequencing, the team tracked the gut microbiome's recovery over six months. The headline finding was that most bacterial species returned within about forty-five days. The less-publicised finding was that nine common species that were present before treatment remained undetectable in most subjects at the six-month mark. They had not recovered. They may never recover [REF:palleja2018].

Sarah's GP refers her to a urologist, who orders more cultures and prescribes a fourth antibiotic — meropenem, a carbapenem, one of the last-resort drugs. This requires intravenous infusion in a hospital outpatient unit. The infection clears. Three months later, it returns. The *E. coli* is now

carbapenem-intermediate. Sarah is running out of drugs, and each course has pushed her further down a narrowing corridor of resistance while progressively degrading the microbial community that, as we discussed in Chapter 5, normally provides colonisation resistance against the very pathogens now overwhelming her.

This is the paradox of empiric antibiotic therapy for chronic infections. The treatment is fast, accessible, and requires no specialised laboratory matching — any GP can write the prescription within minutes. But it is also blunt, ecologically destructive, and self-undermining: each course of broad-spectrum antibiotics selects for the resistant organisms that cause the next infection. For acute, first-episode infections in otherwise healthy people, the system works tolerably well. For chronic, recurrent, or resistant infections, it can feel like trying to put out a fire by pouring petrol on it.

The Phage Pathway: Elena's Story

Elena is fifty-four, a civil engineer from Munich, and she has the same infection — chronic recurrent UTI, multi-drug-resistant *E. coli*, three failed antibiotic courses. Her urologist, having exhausted the formulary, mentions a possibility she has never heard of: phage therapy at the Eliava Phage Therapy Center in Tbilisi, Georgia.

Elena's first step is not getting on a plane. It is getting on the phone. The Eliava center offers both in-person treatment and a distance protocol. She sends her most recent urine culture results to the center — crucially, including the live bacterial isolate, shipped in a transport medium via a medical courier service. This step is essential. Unlike antibiotics, which are prescribed based on the *class* of bacteria causing the infection, phage therapy is prescribed based on the *specific strain*. The phages that will treat Elena must be tested against *her* bacteria, in *her* culture, before a single dose is prepared [REF:zaldastanishvili2021].

At the Eliava laboratory, technicians plate Elena's *E. coli* isolate and test it against the center's standard phage preparations. The Eliava Institute maintains a library of commercial phage cocktails — products with names like Pyo Bacteriophage, Intesti Bacteriophage, and SES Bacteriophage — each containing a mixture of phages active against common pathogens. The testing method is conceptually simple: a drop of phage preparation is spotted onto a lawn of Elena's bacteria. If the phages kill the bacteria, a clear zone — a *plaque* — forms in the bacterial lawn. No plaque, no activity. Plaque means the phage can infect and lyse Elena's strain [REF:zaldastanishvili2021].

In Elena's case, one of the standard preparations shows good activity against her isolate. This is the best-case scenario. If none of the commercial products worked, the center would move to a second step: screening their broader research library of over six hundred characterised phages. If that also failed, they could prepare a *custom* phage — a phage specifically isolated and amplified to target Elena's strain, a process that takes eight to twelve weeks and costs significantly more [REF:zaldastanishvili2021].

Elena decides on in-person treatment and flies to Tbilisi. Her treatment course will last approximately ten to fourteen working days. On arrival, she is evaluated by a physician at the Phage Therapy Center — a clinical consultation that includes a full medical history, review of prior treatments, and fresh specimen collection to confirm the infecting organism has not changed since her samples were shipped.

The treatment itself is multi-route. Elena receives oral phage preparations — taken as a liquid, typically twice daily — for systemic exposure. Because her infection is in the urinary tract, she also receives phages delivered locally: vaginal suppositories containing the same phage preparation,

designed to establish high phage concentrations at the infection site. For prostatitis patients, the center uses rectal suppositories; for respiratory infections, nebulised inhalation. The route matches the anatomy of the infection, a principle that mirrors how any careful clinician chooses between oral and topical antibiotics — but taken further, because phages replicate at the site of infection, amplifying their own concentration precisely where the bacteria are [REF:zaldastanishvili2021] [REF:leitner2021].

What happens during the first week is, from Elena's perspective, somewhat anticlimactic. There is no dramatic crisis, no intensive monitoring. She takes her oral doses, uses the suppositories, and attends periodic check-ups where fresh specimens are collected and cultured. The clinicians are watching for three things. First, is the bacterial load declining? Second, are any new organisms appearing — sometimes, as phages destroy one bacterial population, a previously suppressed species emerges from the biofilm underneath. Third, is the target strain developing phage resistance?

This third question is the critical one, and it is where phage therapy diverges most sharply from the antibiotic model. When bacteria develop antibiotic resistance, the usual response is to switch to a different antibiotic — often a broader-spectrum one, inflicting more collateral damage. When bacteria develop phage resistance, the response is to switch to a different phage — one that targets a different surface receptor on the bacterium. And here is the crucial insight from evolutionary biology: bacteria that evolve resistance to a specific phage often do so by modifying or losing the surface receptor that the phage uses for attachment. But surface receptors are not decorative. They serve essential functions — nutrient uptake, signalling, motility. Losing a receptor to escape a phage frequently comes at a fitness cost. In some well-documented cases, bacteria that evolve phage resistance simultaneously *lose* their antibiotic resistance, because the same surface structures are involved in both. The bacterium trades one vulnerability for another [REF:chan2016].

This phenomenon — sometimes called the “phage resistance trade-off” — is one of the most exciting features of phage therapy from an evolutionary perspective. It means that phage therapy and antibiotic therapy can, in principle, be used in combination to create an evolutionary trap: bacteria that resist the phage become sensitive to the antibiotic, and bacteria that resist the antibiotic remain susceptible to the phage. The organism cannot escape both pressures simultaneously.

Two Timelines, Two Philosophies

The contrast between Sarah's and Elena's experiences illustrates something deeper than a difference in treatment modality. It reflects two fundamentally different philosophies of infectious disease management.

The antibiotic model is *empiric*. It starts from probability: most UTIs are caused by *E. coli*, most *E. coli* are sensitive to trimethoprim, therefore prescribe trimethoprim. It is fast, cheap, and requires no microbiology beyond a standard culture (which, in many healthcare systems, is not even performed for a first uncomplicated UTI). It treats the infection as a generic category — a UTI — rather than as a specific organism in a specific patient. And it works well for first-episode acute infections, which is why it has been the standard of care for decades.

The phage model is *personalised*. It starts from the individual patient's bacterium: isolate it, culture it, test the phages against it, select the ones that work, deliver them by the route that makes anatomical sense, monitor for resistance, adjust as needed. It is slower, more labour-intensive, and requires a level of microbiological engagement that most Western healthcare systems abandoned when antibiotics made it unnecessary. But for the patient whose infection has defeated the

empiric approach — the patient with a multi-drug-resistant chronic infection, the patient who has cycled through four or five antibiotic courses without lasting benefit — the personalised model offers something the empiric model cannot: a treatment matched to *their* infection, not to the statistical average.

The Georgian physicians at Eliava describe their approach as “iterative infectious disease management” — a phrase that captures the difference neatly [REF:zaldastanishvili2021]. Phage therapy is not a one-shot cure. It is a process: test, treat, culture, reassess, adjust. It is closer, in philosophy, to how oncologists manage cancer — with serial monitoring, adaptive treatment plans, and an acceptance that the disease may evolve under therapeutic pressure — than to how most infections are currently treated. It is slower and more demanding. But for the patients who need it, the question is not whether they can afford the time. It is whether they have any alternative.

19.3 Landmark Cases: When Compassionate Use Changed the Conversation

The case that brought phage therapy into the Western medical mainstream was not a clinical trial. It was a desperate gamble made by a wife who happened to be an epidemiologist.

The Patterson Case

In November 2015, Tom Patterson — a sixty-nine-year-old psychiatry professor at the University of California, San Diego — fell critically ill while on holiday in Egypt. He had developed a pancreatic pseudocyst, which became infected with *Acinetobacter baumannii*, a gram-negative bacterium notorious for its ability to acquire resistance genes. Patterson’s isolate was resistant to every available antibiotic. He was medevaced to Frankfurt, then transferred to UC San Diego, where he slipped into septic shock and multi-organ failure. His physicians had nothing left to offer [REF:schooley2017].

His wife, Steffanie Strathdee — a globally recognised HIV epidemiologist — began searching the literature. She found the phage therapy literature and, in a series of frantic emails and phone calls, contacted researchers across the United States. Within weeks, a collaboration had assembled that would have been impossible through normal channels. Teams at Texas A&M University, the United States Navy Medical Research Center, and San Diego State University screened their phage collections against Patterson’s *Acinetobacter* isolate. They found phages that worked. Forest Rohwer’s laboratory at SDSU purified the preparations for intravenous use [REF:schooley2017].

On March 15, 2016, phage therapy began — administered intravenously, directly into Patterson’s bloodstream. Four days later, he opened his eyes and recognised his daughter. It was the first sign of neurological recovery after weeks of unresponsiveness. By June, *Acinetobacter* was undetectable in his body. By August, he walked out of the hospital [REF:schooley2017].

The Patterson case was published in 2017 in *Antimicrobial Agents and Chemotherapy* and became the most widely reported phage therapy story in the Western media. It was not a controlled trial. It was not blinded. Patterson received other treatments alongside the phages. Scientifically, it proved very little in isolation. But it did something more important: it proved that phage therapy could be done — safely, rapidly, in a modern Western hospital, under FDA emergency investigational new drug (IND) authorisation — and that a patient at death’s door could walk out alive.

Strathdee and Patterson later co-authored a book, *The Perfect Predator*, and their case led directly to the founding of the Center for Innovative Phage Applications and Therapeutics (IPATH) at UC

San Diego — the first dedicated phage therapy centre in North America.

The Carnell-Holdaway Case

If the Patterson case demonstrated that phage therapy could work in an emergency, the case of Isabelle Carnell-Holdaway demonstrated something even more remarkable: that phages could be *engineered* for a patient.

In 2017, Isabelle — a fifteen-year-old British girl with cystic fibrosis — underwent bilateral lung transplantation at Great Ormond Street Hospital in London. Post-transplant, she developed a disseminated infection with *Mycobacterium abscessus*, an environmental mycobacterium that is intrinsically resistant to most antibiotics and notoriously difficult to treat. The infection spread to her surgical wound, her skin, and her liver. Her transplant team had exhausted all antibiotic options and, as Isabelle's condition deteriorated, her physicians contacted Graham Hatfull at the University of Pittsburgh [REF:dedrick2019].

Hatfull runs the largest collection of characterised mycobacteriophages in the world — the Science Education Alliance Phage Hunters Advancing Genomics and Evolutionary Science (SEA-PHAGES) programme, built over two decades with the help of thousands of undergraduate students. His team screened Isabelle's *M. abscessus* isolate against the collection and identified three candidate phages. One killed the bacteria efficiently. The other two did not — but Hatfull's team genetically engineered them, deleting the repressor gene that allowed the phages to enter lysogeny rather than killing their hosts, converting them from temperate to obligately lytic. The result was a bespoke three-phage cocktail designed specifically for Isabelle's infection [REF:dedrick2019].

Treatment began in June 2018, with intravenous infusions twice daily and topical application to skin lesions. Within seventy-two hours, the skin sores began to dry. After six weeks, the infection had substantially resolved. The case was published in *Nature Medicine* in 2019 and represented the first therapeutic use of genetically engineered phages in a human patient [REF:dedrick2019].

From Anecdotes to Evidence

These cases — and a growing number like them — are not clinical proof that phage therapy works. They are case reports, the lowest rung of the evidence ladder. The patients were desperately ill, received multiple concurrent treatments, and were not compared to matched controls. Any honest assessment must acknowledge that spontaneous improvement, placebo effect, or synergistic effects of co-administered antibiotics could account for some or all of the observed benefit.

But the case reports have accumulated to a point where the pattern is difficult to dismiss. A landmark 2024 retrospective analysed one hundred consecutive patients treated with personalised phage therapy across thirty-five hospitals in twelve countries. Clinical improvement was observed in 77.2 per cent. Bacterial eradication was achieved in 61.3 per cent. Adverse events were rare — seven non-serious suspected reactions in one hundred cases. Importantly, the study found that bacterial eradication was seventy per cent less likely when phages were given without concurrent antibiotics, suggesting that the combination of phages and antibiotics — the evolutionary trap we described earlier — may be more effective than either alone [REF:dedrick2023].

The evidence is not yet at the level that would satisfy a drug regulator for market approval. No phage therapy product has received full approval from the FDA, the European Medicines Agency, or the TGA. All current use in the West occurs under compassionate access pathways — the FDA's emergency IND mechanism, which can be approved by phone on the same day, or expanded access

protocols for individual patients. Approximately ninety phage therapy clinical trials are now active worldwide, including forty-one in the United States, and the first randomised controlled trials are beginning to report results [REF:fda2023].

But the gap between case reports and regulatory approval is being closed from both ends. From the bottom, the case series are growing larger and more systematically documented. From the top, regulators are developing new frameworks: Belgium has established a manufacturing monograph for phage preparations, Israel has created a “Clinical Phage Microbiology” pipeline analogous to clinical antibiotic susceptibility testing, and the FDA has issued guidance documents on phage therapy as a biological product [REF:fda2023] [REF:yerushalmy2023].

19.4 The Limits of the Comeback

It would be irresponsible to write about phage therapy without writing honestly about its limitations, because the history of medicine is littered with therapies that were oversold before they were understood.

The Georgian model at Eliava — the most mature clinical programme in the world — is itself instructive. The 2021 case series from the Eliava Phage Therapy Center described three patients with chronic bacterial infections treated with phages over extended periods. The results were nuanced, not triumphant. Patient 1, a forty-three-year-old man with cystic fibrosis and chronic *Pseudomonas aeruginosa* lung infection, was treated for four years. He improved — his bacterial load dropped by a factor of ten to one hundred — and he was able to discontinue antibiotics entirely, replacing them with phage monotherapy. But his *Pseudomonas* was never eradicated. Genetic analysis showed that the bacterial population had diversified under phage pressure, with sensitive and resistant strains coexisting. Patient 2, a sixty-four-year-old woman with bronchiectasis, underwent a similar trajectory: five sequential custom phages over three years, each replacing the last as resistance emerged. Her infection eventually cleared, but the path was long and iterative. Patient 3 — a seventy-two-year-old woman with chronic *Klebsiella pneumoniae* cystitis — did not respond. Her bacteria cleared initially but returned within months, and the treatment was ultimately unsuccessful [REF:zaldastanishvili2021].

These outcomes reflect the honest reality of phage therapy. It is not penicillin. It does not reliably eliminate an infection in a single course. It requires ongoing microbiological surveillance, willingness to switch phages as resistance emerges, and acceptance that some infections will not respond. For patients whose infections are otherwise untreatable, a seventy-seven per cent clinical improvement rate is substantial. But it is not a cure rate, and the twenty-three per cent who do not improve — and the thirty-nine per cent whose bacteria are not eradicated even when symptoms resolve — are a reminder that phage therapy is a sophisticated tool, not a magic bullet.

There are also practical barriers. Phage therapy requires microbiology infrastructure that many healthcare systems no longer maintain. It requires phage libraries — large, characterised collections — that take decades to build. It requires clinicians who understand phage biology, something that is not currently part of medical training anywhere in the Western world. And it requires a regulatory framework that does not yet exist for a therapy that is, by design, personalised: every patient potentially receives a different product, which does not fit neatly into the pharmaceutical model of standardised manufacturing and batch testing.

The comparison with Elena’s experience in Tbilisi is illuminating. Elena’s treatment cost approx-

imately €4,200 for a ten-to-fourteen-day in-person course. Whether that is cheap or expensive depends entirely on where you live. In the United States, where healthcare costs are among the highest in the world, a single hospital admission for intravenous antibiotics can easily exceed that amount many times over. In Australia, a public hospital might charge an uninsured foreign patient around AUD 1,300 per day — roughly comparable to the Eliava fee for a short IV course, but potentially exceeding it for a longer one. In many European countries with universal public healthcare, the patient's out-of-pocket cost for hospital-administered antibiotics might be close to zero. The point is not that phage therapy is cheap in absolute terms, but that the cost comparison depends heavily on geography and insurance — and that Elena is paying out of pocket for a treatment that no Western insurer currently covers. She also had to travel to Georgia, wait two to four weeks for an appointment, and entrust her care to a centre that, however experienced, operates outside the regulatory frameworks that Western patients and their physicians are accustomed to. For patients who cannot travel — the elderly, the immunocompromised, those in resource-limited settings — the Georgian model is not accessible. And for healthcare systems that measure quality by standardisation and reproducibility, a therapy that is different for every patient poses challenges that are as much administrative as scientific.

None of these limitations are reasons to dismiss phage therapy. They are reasons to invest in it — in clinical trials, in phage library development, in training programmes, in regulatory innovation. The antibiotic crisis is not going away. The pipeline of new antibiotic classes has produced only a handful of truly novel agents in the past four decades. The bacteria are evolving faster than the pharmaceutical industry can respond. Phage therapy is not the only alternative — antimicrobial peptides, lysins, CRISPR-based approaches, and microbiome engineering (which we will discuss in later chapters) all hold promise. But phage therapy has something the others do not: a century of clinical experience, a living proof-of-concept in Tbilisi, and a growing body of Western case data suggesting that, for the patients who need it most, the viruses that eat bacteria may be the oldest new medicine we have.

In the next section, we will turn to the cutting edge: the engineering of synthetic phages, the design of phage cocktails guided by genomics, and the question of whether phage therapy can ever scale from compassionate use to routine clinical practice.

Key References — Chapter 19

- [REF:dherelle1917] d'Hérelle F (1917). Sur un microbe invisible antagoniste des bacilles dysentériques. *Comptes Rendus Acad. Sci. Paris*, 165, 373–375.
- [REF:kutateladze2010] Kutateladze M & Adamia R (2010). Bacteriophages as potential new therapeutics to replace or supplement antibiotics. *Trends Biotechnol.*, 28(12), 591–595. doi:10.1016/j.tibtech.2010.08.001
- [REF:abedon2011] Abedon ST, Kuhl SJ, Blasdel BG & Kutter EM (2011). Phage treatment of human infections. *Bacteriophage*, 1(2), 66–85. doi:10.4161/bact.1.2.15845
- [REF:chan2016] Chan BK, Siström M, Wertz JE, Kortright KE, Narayan D & Turner PE (2016). Phage selection restores antibiotic sensitivity in MDR *Pseudomonas aeruginosa*. *Sci. Rep.*, 6, 26717. doi:10.1038/srep26717
- [REF:schooley2017] Schooley RT, Biswas B, Gill JJ et al. (2017). Development and use of personalized bacteriophage-based therapeutic cocktails to treat a patient with a dissemin-

- ated resistant *Acinetobacter baumannii* infection. *Antimicrob. Agents Chemother.*, 61(10), e00954-17. doi:10.1128/AAC.00954-17
- [REF:palleja2018] Palleja A, Mikkelsen KH, Forslund SK et al. (2018). Recovery of gut microbiota of healthy adults following antibiotic exposure. *Nat. Microbiol.*, 3(11), 1255–1265. doi:10.1038/s41564-018-0257-9
 - [REF:dedrick2019] Dedrick RM, Guerrero-Bustamante CA, Garlena RA et al. (2019). Engineered bacteriophages for treatment of a patient with a disseminated drug-resistant *Mycobacterium abscessus*. *Nat. Med.*, 25(5), 730–733. doi:10.1038/s41591-019-0437-z
 - [REF:zaldastanishvili2021] Zaldastanishvili E, Leshkasheli L, Dadiani M et al. (2021). Phage Therapy Experience at the Eliava Phage Therapy Center: Three Cases of Bacterial Persistence. *Viruses*, 13(10), 1901. doi:10.3390/v13101901
 - [REF:dickstein2023] Dickstein Y, Jeong JY, Brintz BJ et al. (2023). Antibiotic resistance in urinary tract infections: results from a multi-site cohort. *J. Infect. Dis.*, 230(6), e1344. doi:10.1093/jid/jiad123
 - [REF:dedrick2023] Dedrick RM, Smith BE, Cristinziano M et al. (2023). Personalized bacteriophage therapy outcomes for 100 consecutive cases: a multicentre, multinational, retrospective, observational study. *Nat. Microbiol.* doi:10.1038/s41564-024-01705-x
 - [REF:leitner2021] Leitner L, Ujmajuridze A, Chanishvili N et al. (2021). Intravesical bacteriophages for treating urinary tract infections in patients undergoing transurethral resection of the prostate: a randomised, placebo-controlled, double-blind clinical trial. *Lancet Infect. Dis.*, 21(3), 427–436. doi:10.1016/S1473-3099(20)30330-3 [Published online 2020; print March 2021]
 - [REF:fda2023] U.S. Food and Drug Administration (2023). Science and Regulation of Bacteriophage Therapy. FDA Guidance Document.
 - [REF:yerushalmy2023] Yerushalmy O, Khalifa L, Copenhagen-Glazer S et al. (2023). Towards Standardization of Phage Susceptibility Testing: The Israeli Phage Therapy Center “Clinical Phage Microbiology” — A Pipeline Proposal. *Clin. Infect. Dis.*, 77(Suppl 5), S337–S351. doi:10.1093/cid/ciad514
 - [REF:aswani2021] Aswani VH & Shukla SK (2021). An Early History of Phage Therapy in the United States: Is It Time to Reconsider? *Clin. Med. Res.*, 19(2), 82–89. doi:10.3121/cmr.2021.1605

Chapter 20: Diet — Feeding the Ecosystem

20.1 The Dry Season in Hadzaland

In the acacia woodlands south of Lake Eyasi, in northern Tanzania, a group of people still live in a way that most of humanity abandoned about ten thousand years ago. The Hadza are among the last full-time hunter-gatherers on earth. There are perhaps a thousand of them, and only a few hundred still live almost entirely on what they can hunt, dig, and pick from the landscape around them. They do not keep animals. They do not plant crops. They do not have a word for “farmer.” What they eat today is what they found today.

In 2013 and 2014, a team of researchers from Stanford University and the University of Bologna went to live with the Hadza and do something that had never been done before: follow the same people, through an entire year, and track what happened inside them as the seasons turned. They collected stool samples in the wet season, when the women’s foraging brought back baobab pods, berries, and tubers in abundance, and the men returned with honey stolen from wild bee hives high in the acacia trees. Then they collected samples again in the dry season, when the berries were gone, the tubers were woody and bitter, and the camp subsisted mostly on meat and *ekwa*, a hard root dug out of the cracked earth with pointed sticks [REF:smits2017].

What they found was astonishing. The gut microbes of the Hadza did not stay the same. They *cycled*. Entire families of bacteria that were abundant in the wet season almost disappeared in the dry season, and then came back when the rains returned and the fruits and tubers followed. One group, the Bacteroidetes — a family we have met many times in this book — surged when the diet was high in plant fibre and retreated when it was not. Another group, the Firmicutes, moved in the opposite direction. The researchers were watching, in real time, a rainforest of bacteria change species composition with the weather.

Now compare that with the microbes of a person in Melbourne, Manchester, or Minneapolis. Here the seasons, such as they are, do not reach the supermarket. A tomato in January tastes the same as a tomato in July, only worse. The diet does not cycle. The microbes do not cycle. Or rather — and this is the more disturbing finding from a different branch of the same research program — they have already stopped cycling. Many of the taxa that cycle in the Hadza are entirely absent from the Western gut. We are not in a permanent summer. We are in a permanent winter so long that most of us no longer remember anything else [REF:sonnenburg2016] [REF:schnorr2014].

This chapter is about that forgotten summer — and about how, three times a day, you get a vote on whether it returns.

20.2 The Gardener’s Dilemma

In Chapter 18 we talked about antibiotics as something closer to a forest fire than to a scalpel — a tool that treats the infection and sterilises the ground around it. In Chapter 19 we talked about

phages, which act more like a sniper than a flame-thrower. Both chapters were about killing things. This chapter is about feeding them.

The distinction matters because almost everything the popular science shelf of a health food store sells you is framed as a killing or a planting intervention. “Cleanses” kill. “Probiotics” plant. Both framings are, for most people most of the time, the wrong way to think about the problem. Your microbiome is not a garden in the sense that you start from bare earth and decide what goes where. By the time you are old enough to read a label on a supplement bottle, the garden is decades old. Some of your microbial species have been with you since you came down the birth canal. Others joined when you were weaned, or when you went to primary school, or when your family moved house. Many of them cannot be bought in a capsule at all. Some of them are probably extinct in most of your neighbours, and you will not get them back by swallowing a pill.

What you *can* do, every day, is decide what to feed them.

The metaphor to hold in mind for the rest of this chapter is not pharmacology. It is agriculture. A gardener does not usually plant a new lawn every spring; she feeds the one she has. She chooses composts and mulches that favour certain plants and disadvantage others. She waters in patterns that support the soil she wants, and she knows that what she cannot see — the fungi and bacteria and protists underneath — is doing more of the work than the bits she can. Over years, her choices produce a particular kind of garden. Somebody else, making different choices with the same starting soil, produces a different one. Neither of them got to pick the seeds.

This chapter is a practical guide to feeding the garden you have. It is organised around a question the reader is entitled to ask at the end of any chapter on diet: *so what should I eat on Tuesday?* We will get to a direct answer, tiered by the strength of the evidence, in section 20.12. Before we get there, we have to understand what we are feeding, what the microbes do with what we give them, and why the answers are not always the same for every gardener.

We will work outwards from the strongest and cleanest evidence — fibre — to progressively more complex and contested territory: fermented foods, polyphenols, the Mediterranean pattern, protein and fat, ultra-processed food, meal timing, personalisation, and the controversies (gluten, FODMAPs, lectins, carnivore) that readers will have encountered on social media and are entitled to have addressed directly. Nothing in this chapter is invented. Every claim is tied to a published study, and where the evidence is weak or preliminary I will say so plainly.

One warning before we begin. The history of nutrition science is littered with confident claims that did not survive the next decade. Eggs were bad, then good, then bad, then neither. Fat was the enemy, then sugar was the enemy, then insulin, then inflammation. A reasonable reader, watching that parade go by, might conclude that nutrition science is simply unreliable and the sensible thing to do is ignore it. That conclusion would be wrong, but it would be understandable. What I can promise is this: the evidence we will draw on in this chapter is *microbiome-mediated* evidence, which is a different kind of evidence from the older generation of “association studies” that drove the older reversals. We can now watch, in human subjects, what happens to specific microbial populations, specific metabolites, specific inflammatory markers, and in some cases specific clinical outcomes when the diet changes. The evidence is not perfect. But it is less likely to reverse, because it is grounded in a mechanism that can be observed directly.

With that, let us look at the most important thing any human being can put on the plate for the ecosystem inside them: fibre.

20.3 The Currency of the Realm: Fibre and the Microbiota-Accessible Carbohydrates

What your body cannot digest, the microbes can

When you eat a piece of wholemeal toast, your digestive enzymes break down most of it. The starches in the flour are split into glucose and absorbed in the small intestine. The proteins are cleaved into amino acids. The fats, if there are any, are emulsified by bile and picked up by the enterocytes that line the small bowel. By the time what remains of the toast reaches the large intestine, the components that your own enzymes know how to handle are gone.

What is left is the part of the plant that your body cannot digest. The botanical name for most of it is dietary fibre. The chemical reality is more interesting. “Fibre” is not one molecule. It is a bag of very different polysaccharides — cellulose from plant cell walls, hemicelluloses from the matrix that holds those walls together, pectins from the soft tissues of fruits, inulin from the roots of chicory and the bulbs of onions, resistant starch from cooked-and-cooled potatoes and unripe bananas, beta-glucans from oats and barley, arabinoxylans from wheat bran. Some of these you have heard of. Most of them you haven’t. What they have in common is that the human genome does not encode the enzymes to take them apart.

The microbes in your large intestine do. This is one of the most important biochemical facts in this entire book. Your genome carries instructions for making roughly seventeen digestive enzymes that act on carbohydrates. The combined genome of the microbes in your colon encodes tens of thousands of such enzymes, organised into complex multi-protein machines called carbohydrate-active enzymes, or CAZymes. The gut is, in effect, a second digestive system — one that runs on different software than your own, and that is capable of metabolising substrates your own system cannot touch [REF:koh2016].

In 2014, Erica and Justin Sonnenburg at Stanford proposed a name for the subset of dietary carbohydrates that reach the large intestine and feed this second digestive system: microbiota-accessible carbohydrates, or MACs [REF:sonnenburg2014]. The name sounds technical, but the concept is the single most important idea in modern dietary microbiology, and once you see it you cannot unsee it. Every meal you eat delivers some amount of MAC to your colon. The higher the MAC content of your meal, the more substrate your microbes have to work with. The lower the MAC content, the more your microbes have to find something else to eat — and as we are about to see, they do not starve quietly.

The mucus is also food

The human large intestine is lined with a layer of mucus that is not decorative. It is a physical and chemical barrier, two layers thick in the colon, that keeps the trillions of resident bacteria in the lumen from touching the epithelial cells underneath. The outer layer is loose and colonised by bacteria that graze on it. The inner layer is dense, highly cross-linked, and — in health — virtually sterile. The cells in the epithelium sit under a shield of what is, for the bacteria that grow above it, the last line between them and host tissue. This matters enormously because the moment bacteria reach the epithelium, the immune system notices, and inflammation follows.

In 2016, a team led by Mahesh Desai, working in the laboratory of Eric Martens at Michigan, put gnotobiotic mice — mice with a defined, human-derived gut community — through a controlled dietary experiment. One group got a diet containing plenty of plant polysaccharides. The other got a diet essentially devoid of fibre but nutritionally complete in every other respect. The team then

used fluorescent probes to measure the thickness and composition of the colonic mucus layer, along with metagenomics to see what the bacteria were doing [REF:desai2016].

The finding was so vivid it made the cover of *Cell*. On the fibre-free diet, the mucus layer thinned dramatically. The bacteria, unable to find the plant polysaccharides they were equipped to degrade, switched their metabolism to the only remaining source of carbohydrate in the bowel — the glycans of the host mucus layer itself. Specific species that carry the enzymatic machinery for mucin degradation, including *Akkermansia muciniphila* and *Bacteroides caccae*, bloomed. The bacteria were literally eating the wallpaper. Worse, when the mice were then challenged with a pathogen — *Citrobacter rodentium*, a rough mouse equivalent of enterohaemorrhagic *E. coli* — the fibre-deprived animals developed more severe disease than the fibre-fed ones. The thinned mucus layer had become a highway rather than a barrier.

Think about what that finding means. If you stop feeding your microbes fibre, they don't quietly shrink away. They pivot — and start eating *you*. The mechanism is not unique to mice. Mucin-degrading bacteria are abundant in the human colon, and the machinery they use to take mucus apart is, in biochemical terms, general-purpose. The experiment is hard to replicate in humans for ethical reasons, but the implication is serious: the thickness of the mucus layer that separates our bacteria from our own cells is, in part, a function of how much fibre we eat.

What your grandchildren will inherit

A second Sonnenburg paper, also in 2016, made the stakes even clearer. Using the same kind of mice carrying a human-derived microbiota, the team put animals on a low-MAC diet for a single generation and measured what happened to the next [REF:sonnenburg2016].

The parents lost some of their bacterial diversity on the low-MAC diet — roughly a 60% reduction in the species richness of their gut community. When they were put back on the high-MAC diet, most of what they had lost came back. Not all of it, but most. But when the mice were bred, the pups inherited only the species that had survived the low-MAC period. The pups never encountered the missing species and therefore never acquired them. By the fourth generation on the cycling diet, many of those species were gone entirely. Even when the grandchildren and great-grandchildren were put back on a high-MAC diet, the species did not come back. They were extinct.

The experiment is in mice, and we should be careful about what it predicts for humans. But the logic of extinction applies to any transmitted microbial community. If a mother carries species A, B, and C, and her child is born and breastfed, the child acquires species A, B, and C. If the mother has lost species C entirely before conception, the child cannot inherit what is not there. Multiple generations of low-fibre eating in a population can, in principle, produce exactly the pattern Sonnenburg's mice showed: a cumulative, generation-over-generation loss of diversity that does not bounce back even when diet improves, because the species simply are no longer in the transmission pool.

Look at the Western microbiome. Compared with the microbiomes of traditional populations — the Hadza, the Yanomami of the Amazon, the Matsigenka of the Peruvian jungle, rural Malawian children — it is strikingly impoverished. Between a third and a half of the bacterial species that those populations carry are simply not present in people who grew up in industrialised cities. We used to think the explanation was environmental exposure: that hunter-gatherers pick up more microbes from dirt, water, and animals than city dwellers do. The Sonnenburg mouse experiment adds another explanation, one that is harder to hear. We may have eaten some of them into extinction, across generations, without realising it [REF:sonnenburg2016].

This is why fibre deserves its place at the start of this chapter. It is not, as some decades of nutritional advice framed it, merely useful for keeping things moving. It is the substrate that keeps the entire bacterial community alive and operational in its normal configuration. Stop supplying it, and the community does not pause politely. It eats the house, loses members, and in the long run — if the pattern is maintained across generations — it loses them permanently.

What the microbes give back: short-chain fatty acids

When microbes ferment MACs — and “fermentation” here means anaerobic breakdown by bacterial enzymes, not the pickle-jar kind, which we will get to in section 20.6 — they produce a small number of remarkable molecules called short-chain fatty acids, or SCFAs. Three of them dominate: acetate (two carbons), propionate (three carbons), and butyrate (four carbons). They are produced in roughly a 60:20:20 ratio in a healthy adult colon, and between them they account for somewhere between 5 and 10 percent of the total daily energy intake of the host [REF:koh2016].

Each has a distinct job.

Butyrate is the most remarkable, because it is the primary energy source for the cells lining your own large intestine. The colonocytes — the epithelial cells of the colon — get roughly 70% of their energy from bacterial butyrate rather than from the blood supply. This is one of the most intimate metabolic relationships between any two kingdoms of life. You are not running your own colon on your own fuel. The bacteria that sit in the lumen are, quite literally, feeding the cells that face them. When butyrate production drops — because MAC intake drops, because the fibre-fermenting bacteria are not there, because antibiotics have killed them — the colonocytes fall back on less favourable fuels, and the tight junctions between them begin to loosen. The permeability of the gut wall, in other words, is partly a function of how well-fed the colonocytes are, and the colonocytes’ feeding is partly a function of how much fibre you ate yesterday.

Butyrate does more than feed cells. Inside the colonocyte, it acts as a signalling molecule. It inhibits an enzyme called histone deacetylase, or HDAC, which changes how certain genes are read. One of the downstream effects is the differentiation of a particular class of immune cells — regulatory T cells, or Tregs — that keep the colonic immune system from over-reacting to the bacteria it lives with. In 2013, two separate groups published almost simultaneously in *Nature* and in *Science* showing that butyrate drives Treg differentiation in the colon and suppresses colitis in mouse models [REF:furusawa2013] [REF:smith2013]. The food you eat, via the microbes that eat it, signals to the cells that decide whether your immune system tolerates what it sees in your gut or attacks it.

Propionate has its own story. Much of what the bacteria produce is absorbed into the portal vein and taken directly to the liver, where it influences gluconeogenesis — the making of new glucose — and lipid synthesis. Propionate appears to reduce appetite in some studies by acting on gut hormones like PYY and GLP-1, the same hormones targeted by the latest generation of weight-loss drugs. It is not a drug. But it is an endogenous molecule produced, by your bacteria, in proportion to the fibre you feed them.

Acetate is the most abundant and the most diffusible. It reaches the systemic circulation and is taken up by peripheral tissues, including muscle and adipose tissue. Its signalling role is less well understood than butyrate’s, but it contributes to the cross-talk between the gut and brain and appears to play a role in appetite regulation.

All three of them — acetate, propionate, butyrate — act, collectively, as chemical messengers that your microbes send to your cells. They are the receipt your microbes give you in exchange for the

fibre you supply. Low fibre in, low SCFAs out. And low SCFAs out means a colon that is both hungrier and more inflamed than it should be.

The hard outcomes

If the mechanism were all we had, a cautious reader would be right to ask whether it matters clinically. Cells in petri dishes do all sorts of things they do not do in living people, and mouse experiments do not always translate to the human bowel. The honest answer is that we also have clinical outcome data, and it is striking.

In 2019, a consortium led by Andrew Reynolds at the University of Otago in New Zealand published, in *The Lancet*, a series of systematic reviews and meta-analyses pooling data from 185 prospective cohort studies and 58 randomised controlled trials — nearly 135 million person-years of observation [REF:reynolds2019]. The question they asked was simple: does carbohydrate quality, and specifically dietary fibre intake, predict hard clinical outcomes?

The answer was yes, and the magnitude was larger than most people realise. For every eight grams per day of additional fibre intake, the analysis found an 8% reduction in all-cause mortality, a 7% reduction in coronary heart disease, a 19% reduction in colorectal cancer, and a 15% reduction in type 2 diabetes. The dose-response was continuous: more fibre, less disease, all the way up to the highest intake categories — about 25 to 29 grams per day — with no plateau in the data at conventional intake levels and suggestions that higher might be better still.

For context, the average daily fibre intake in most Western countries is around 12 to 15 grams. The recommendations from most national dietary guidelines are 25 to 30 grams per day for adults. The intake of traditional populations has been estimated at around 100 grams per day — roughly seven times what most of us manage. The Hadza, in the wet season, eat around 150 to 200 grams of fibre per day, because baobab pods and wild tubers are essentially concentrated fibre delivery devices. You would not enjoy eating what the Hadza eat. But the gap between their intake and ours is the gap between a microbiome that cycles through seasons and one that has forgotten what a season is.

The Reynolds analysis is not the only evidence, but it is the cleanest recent summary, and it gives us a number to carry forward: eight grams of fibre per day buys you roughly an 8% reduction in your risk of dying of anything. That is an extraordinary number for any dietary variable, and it is backed by the largest and most rigorous synthesis in the field.

The many shapes of fibre

One more thing before we move on, because “just eat more fibre” is not a useful instruction without some feel for what the word actually means.

Fibre is not one thing. The commercially useful distinction is between *soluble* and *insoluble* fibre, based on whether the fibre dissolves in water, but this distinction is a legacy of food chemistry, not microbial biology, and it maps only loosely onto what the microbes actually care about. A more useful breakdown, for our purposes, is functional:

Fermentable fibres are the ones the microbes can take apart readily and use as fuel. Inulin (in chicory root, Jerusalem artichoke, garlic, onion, leek, asparagus), resistant starch (in cooked-and-cooled potatoes, cooked-and-cooled rice, green bananas, and legumes), beta-glucans (in oats and barley), and pectins (in apples, citrus peel, and most fruits) fall into this category. These are the ones that generate the most SCFAs and feed the most microbes.

Slowly fermentable fibres — arabinoxylans in wheat bran, certain hemicelluloses in whole grains — are broken down more gradually and tend to be fermented further along the colon, producing SCFAs in regions where the first category has mostly been consumed. This matters because cancer risk in the colon is highest in the distal portion, and SCFA production in that area depends on slowly fermentable fibres that survive the early part of the journey.

Poorly fermentable fibres — cellulose and lignin, the structural fibres of plant cell walls and wood — are largely excreted intact. They have important non-microbial effects on bowel transit and stool bulk. But they do not, as such, feed the ecosystem.

A good diet contains all three classes. If you eat a bowl of oats topped with berries and a spoon of ground flax in the morning; some lentils or chickpeas and leafy greens at lunch; and a dinner of whole-grain pasta with vegetables, herbs, olive oil, and a small piece of fish, you have covered the full spectrum without thinking about it. If you eat white toast, a ham sandwich on white bread, and pasta with tomato sauce, you have not.

The 2024 review by Deehan and colleagues is the best single summary of fibre subtypes and their metabolic effects currently available, and readers who want the biochemical detail can find it there [REF:deehan2024]. For our purposes, the single most important take-away from the fibre section of this chapter is this: your microbes run on substrate, the substrate is fibre, and there are a great many different fibres with subtly different effects. The next section turns that insight into an actionable rule — a single number that, of everything in this chapter, is probably the most concretely useful thing the modern microbiome literature has produced.

20.4 Diversity Beats Dose: The Thirty-Plant Rule

A number you can actually use

If this chapter had to offer the reader one number, and only one, the number would be thirty.

It comes from the American Gut Project, a citizen-science study that, between 2012 and 2017, enrolled more than 11,000 volunteers in the United States, the United Kingdom, and Australia. Participants paid a small fee, were mailed a swab kit, sampled their own stool, and filled in a long dietary questionnaire. The samples were sequenced at the laboratory of Rob Knight at the University of California San Diego. The scale of the project — roughly an order of magnitude larger than any comparable microbiome study at the time — allowed the analysts to ask questions that smaller studies cannot answer, including: which dietary variable, out of dozens tracked, best predicts the diversity of the gut microbiome? [REF:mcdonald2018]

The answer was not total fibre grams. It was not whether the participant identified as vegan or vegetarian. It was not meat consumption, carbohydrate consumption, or calorie intake. It was a single variable the research team called “plant types per week”: the number of distinct plant species a person reported eating over the previous seven days.

The result fell cleanly into two groups. People who reported eating thirty or more different types of plants per week had, on average, significantly more diverse gut microbiomes than people who reported ten or fewer. The effect was dose-dependent, consistent across the three countries, and robust to adjustment for fibre intake, age, body mass index, and whether the person called themselves a plant-eater. Eating more *species* of plants, regardless of how much fibre was in any single serving, predicted a richer ecosystem in the gut.

Thirty plant species a week sounds like a lot. Most people, asked to guess how many they eat, guess something like ten or fifteen. Almost everybody underestimates. The trick is what you count. Every plant counts: fruits, vegetables, whole grains, legumes, nuts, seeds, herbs, and spices. A salad with mixed greens, tomato, cucumber, red onion, parsley, and a dressing of olive oil, lemon, and black pepper contains eight different plants before you have finished chewing. A bowl of muesli with oats, wheat flakes, barley, almonds, walnuts, sunflower seeds, flaxseed, dried cranberries, and raisins is nine. A good Indian dal with lentils, tomato, onion, garlic, ginger, turmeric, cumin, coriander, chilli, and a wedge of lime is ten. A piece of dark chocolate counts. Coffee counts. Green tea counts. Even a sprinkle of basil on top of a dish counts.

Once you start keeping track, thirty a week is not only achievable — it is surprisingly easy for anyone who cooks at home and uses a spice rack. For the person who lives on sandwiches, ready meals, and takeaway, it is almost impossible. That asymmetry is the most honest summary of what the modern microbiome literature is telling us about how food choices matter at the everyday level.

Why diversity, not dose

The why behind the thirty-plants finding is worth pausing over, because it tells us something about the nature of the gut ecosystem that the fibre story alone does not.

Each plant species contains a somewhat different mix of polysaccharides, polyphenols, proteins, and minor bioactive molecules. A carrot and a parsnip look similar on the plate but carry different classes of polysaccharide in their cell walls. Oats deliver beta-glucans that barley has less of; barley delivers arabinoxylans that oats have less of. A blueberry carries a different polyphenol profile from a blackberry, and both differ from a strawberry. Each of these slightly different substrates is best handled by a slightly different subset of gut microbes — different enzymes, different species, sometimes different whole metabolic guilds.

A person who eats the same four or five plants every day has, in effect, been running a one-crop farm in their colon. The microbes that can handle those four or five crops thrive. The microbes that require something else — a different polysaccharide, a different polyphenol, a different trace amount of some obscure glycan — cannot find work, and their populations dwindle. Over months and years, the ecosystem contracts around the narrow diet.

A person who eats thirty or forty plants a week is running something more like a mixed polyculture. Nothing is in huge abundance, but almost every microbial guild finds something to do. The ecosystem holds its diversity not because any one food is magical but because the *variety* creates niches for a wider range of inhabitants.

This is not speculation. A series of studies has now shown that increasing the *variety* of fibre types in a diet — rather than the total quantity — changes the microbiome and the metabolome in measurable ways. Ranaivo and colleagues, in a 2022 trial, showed that enriching a daily-consumed bread with a diverse rather than uniform mixture of fibre sources produced larger changes in microbial composition and metabolic markers in subjects at cardiometabolic risk, even when the total fibre dose was held constant [REF:ranaivo2022]. Diversity, in other words, is doing work that quantity alone cannot replicate.

The practical advice that falls out of this is refreshingly unlike most nutritional advice, because it does not require you to eliminate anything. It just asks you to add. When you plan a shopping list, reach for one new vegetable you have not bought before. When you cook, reach for one more herb than the recipe calls for. Keep seeds (sesame, pumpkin, sunflower, flax, chia) in the pantry

and sprinkle them on things. Eat the skins of fruits when they are edible; keep the leaves on the beetroot; buy whole spices and grind them yourself. Every species added is a new niche opened inside you.

Two things you can't see on the plate

There is a further reason to favour variety — two of them, in fact — that the thirty-plants number happens to hedge against even though the American Gut investigators were not looking for them. Both have to do with the quiet fact that the food on the modern plate is not quite what the food on the same plate was a few decades ago.

The first is what agronomists sometimes call the *dilution effect*. Donald Davis and colleagues at the University of Texas compared United States Department of Agriculture food-composition tables for forty-three garden crops between 1950 and 1999 and found statistically reliable declines in six of thirteen nutrients — protein, calcium, phosphorus, iron, riboflavin and ascorbic acid — on the order of six to thirty-eight per cent. The authors were careful in their own interpretation: the declines, they wrote, are “most easily explained by changes in cultivated varieties between 1950 and 1999, in which there may be trade-offs between yield and nutrient content” [REF:davis2004]. In other words, the same tomato or head of lettuce on today's supermarket shelf is very likely a different *cultivar* from the one your grandmother bought, bred over decades to pack more water, more starch, more sheer weight into each fruit, and proportionally less of several minerals and vitamins. The effect is not catastrophic and does not apply to every crop or every nutrient, but it is real enough, and consistent enough across foods, that it is difficult to dismiss. And unlike flashier concerns, it is quietly cumulative: a diet of five plants whose mineral density has slipped a third over two generations simply supplies less of those minerals, full stop.

Whether the drift matters for the microbiome is a reasonable next question, and the honest answer is: at the margin, in ways we are still learning to measure, probably yes. Gut bacteria have their own trace-element requirements, and the community does not merely follow the host's mineral status but actively shapes it. A 2021 review by Bielik and Kolisek catalogued the two-way traffic — dietary minerals shape which microbes thrive, and microbial metabolism in turn influences how much of those minerals the host can absorb [REF:bielik2021]. The surprising twist is that simply topping up a deficient nutrient in isolation does not automatically restore a healthy ecosystem, and can even make things worse. In a series of randomised trials in Kenyan infants, iron-fortified porridge reduced anaemia — a genuine benefit — but also shifted the colon toward more *Enterobacteriaceae* (including pathogenic *Escherichia coli*), reduced bifidobacteria, raised a biomarker of enterocyte damage, and increased treated respiratory infections. Adding a prebiotic (galacto-oligosaccharides) alongside the iron cancelled most of these adverse effects [REF:paganini2017]. A follow-up study in the same population showed that iron fortification even blunted the response to oral antibiotics and raised the risk of post-antibiotic diarrhoea [REF:paganini2018]. A broader review of excess iron in infants and young children reaches a consistent conclusion: iron is helpful when deficient, pro-inflammatory and dysbiotic when in surplus, and the margin between the two is narrower than many public-health programmes assume [REF:lonnerdal2017]. The lesson is not that fortification is bad — it is that a nutrient arriving on its own, without the food matrix it evolved inside, behaves differently from the same nutrient arriving embedded in a plant. The safer strategy — the one that captures whatever micronutrients our soils and cultivars still deliver, spreads the bet across many sources, and avoids accidental megadoses — is once again the thirty-plant rule.

The second hidden variable is residue. Of all the pesticides in routine modern use, the herbicide

glyphosate is the one the microbiome literature has scrutinised most closely, because its mechanism of action is not incidental to bacteria but directly relevant to them. Glyphosate kills plants by inhibiting a single enzyme, 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), which sits at the heart of the *shikimate pathway* — the route by which plants, fungi, and many bacteria build the aromatic amino acids tryptophan, tyrosine and phenylalanine. Humans lack the shikimate pathway entirely; we obtain these amino acids from food. This is the long-standing argument that glyphosate is safe for mammals. The catch, however, is that a large fraction of our gut bacteria *do* possess the pathway, and whether any particular species is sensitive or resistant depends on which *class* of EPSPS enzyme it carries. In honey bees, whose gut community is unusually tractable, Motta and colleagues showed that chronic exposure at environmentally realistic doses reshaped the community in a way that mapped precisely onto this distinction: species with the sensitive enzyme class declined, those with the resistant class held their ground, and the exposed bees became more susceptible to opportunistic infection [REF:motta2018]. The mechanism, in short, is not disputed.

Whether the doses that reach the human colon through food residue are sufficient to reproduce such an effect in a mammal is less settled, but the evidence is no longer nothing. In 2021, Mesnage and colleagues fed rats either glyphosate or the commercial Roundup formulation MON 52276 at doses bracketing the European regulatory limit, and combined shotgun metagenomics of the caecum with serum and caecal metabolomics. Both treatments caused shikimic acid and 3-dehydroshikimic acid — the substrates that accumulate when EPSPS is blocked — to build up in the gut contents, providing the first direct *in vivo* biochemical evidence that the shikimate pathway of the mammalian gut microbiome is in fact inhibited at doses within the legal range [REF:mesnage2021]. An earlier Ramazzini Institute study, using the United States Environmental Protection Agency's acceptable daily intake as the reference dose, found microbial and reproductive changes in the pups of rats whose mothers had been exposed during pregnancy [REF:mao2018]. The picture, however, is not one of straightforward devastation, and it would be dishonest to pretend otherwise. A 2022 biomonitoring study of British twins, which detected glyphosate in the urine of more than half of adult participants, actually found that higher excretion was weakly associated with *greater* microbial richness, alongside changes in several bacterial metabolites — a reminder that the effects are subtle, probably diet-dependent, and not a simple linear “more glyphosate, worse gut” story [REF:mesnage2022twins]. A 2023 review in *Gut Microbes* by Walsh and colleagues summarised the field as consistent with biologically plausible effects on community composition at realistic exposures, with genuine uncertainty about long-term and developmental outcomes [REF:walsh2023]. The appropriate response is neither panic nor dismissal. It is, once again, the ecological one: eat a wide enough variety of plants, from a wide enough variety of sources, that no single residue dominates the exposure — and, where the option is available and affordable, prefer sources (organic, small-farm, home-grown, seasonal) on which glyphosate is less likely to have been used in the first place.

Both of these stories — the dilution effect and the residue effect — are stories about things the modern eater cannot directly see or taste, and cannot realistically measure. Neither on its own is a crisis. But both are reasons why the answer to “how do I protect my microbiome from things I cannot even detect?” turns out to be the same as the answer to “how do I feed my microbiome well?” A diet of thirty different plant species a week is not a guarantee against either problem, but it is the simplest available hedge: it spreads the load across many sources, increases the chance that at least some of what you eat carries the minerals the tables promised, and dilutes the contribution of any single contaminated supply. Diversity, once again, is doing work that no single “superfood” and no supplement can replicate.

The redundancy that keeps the ecosystem upright

There is a second reason diversity matters that applies to ecosystems in general, including the one inside you: redundancy is resilience.

A forest with two dozen tree species is not only richer than a forest with three. It is also harder to destroy. If a pathogen arrives that attacks one species, the other twenty-three keep the forest standing. If a drought kills the shallow-rooted trees, the deep-rooted ones remain. The ecological jargon for this is “functional redundancy” — more than one species performs each key function, so losing any one member does not collapse the function.

The same holds for the gut. The production of butyrate, for example, is performed by a consortium of species — *Faecalibacterium prausnitzii*, *Roseburia intestinalis*, *Eubacterium rectale*, and many others. In a diverse microbiome, if one is temporarily suppressed (by an antibiotic, say, or a bout of illness), the others keep producing butyrate. In a monoculture gut where only one butyrate producer survives, losing that species means losing the function — and losing the SCFA means losing the colonocyte fuel and the Treg signal and the tight-junction integrity, all at once.

We cannot yet measure each reader’s redundancy directly. But we can predict with confidence that a gut supplied with thirty kinds of plant substrate a week will have more functional redundancy than one supplied with five. The thirty-plant rule is, in the end, a rule about building an ecosystem that can take a hit and stay upright.

20.5 The Mediterranean Pattern, Put to the Test

Why this one and not the others

The dietary patterns shelf of any bookshop is a chaos of competing claims. Keto, paleo, Whole30, DASH, Zone, Atkins, South Beach, carnivore, alkaline, blood-type, Gundry, Mediterranean, Nordic, Okinawan, MIND, intermittent fasting, flexitarian, and a dozen more — each with adherents, testimonials, and books that promise that this one, at last, is the answer.

Of all of them, the Mediterranean pattern is the only one that has been subjected, over decades, to the kind of testing that clinical medicine applies to a new drug: long-term randomised controlled trials with hard clinical endpoints, conducted in multiple countries, replicated across age groups, and scrutinised by independent review. It is also — and this is not a coincidence — the one that accidentally aligns most closely with the principles this chapter has been building up.

I say “accidentally” because the Mediterranean diet was not designed to feed a microbiome. It was observed in the mid-twentieth century by the American physiologist Ancel Keys, who noticed that people living on the Greek island of Crete and in certain regions of southern Italy had strikingly low rates of coronary heart disease despite eating enough calories and enough fat. Keys characterised what they were eating — plenty of vegetables, fruits, legumes, whole grains, nuts, olive oil, moderate amounts of fish, small amounts of meat and dairy (much of it fermented), modest amounts of wine with meals — and his characterisation became the template for the “Mediterranean diet” as it is now understood. The microbiome science that would eventually explain *why* it worked was still fifty years away.

What the pattern looks like on a plate is not exotic. It is mostly plants, in wide variety, lightly processed, eaten slowly, with olive oil as the dominant fat, and a small portion of animal protein

somewhere in the meal. It is Tuscan ribollita, Greek horiatiki, Provençal ratatouille, Lebanese fattoush, Turkish mezze. It is vegetables that taste like vegetables and bread that tastes like bread. A reader from almost any culture can find a version of it in their own food tradition.

NU-AGE: the big elderly trial

The most compelling recent evidence that the Mediterranean pattern works *through the microbiome* came from a study called NU-AGE, published in 2020 by Tarini Ghosh and Ian Jeffery at University College Cork working with collaborators across five European countries [REF:ghosh2020].

NU-AGE was a one-year randomised controlled trial in 612 elderly participants (aged 65 to 79) from Italy, France, the Netherlands, the United Kingdom, and Poland. The intervention group received personalised dietary counselling and tailored food deliveries to help them shift their eating towards a Mediterranean pattern. The control group continued their usual diet. At the end of the year, the researchers sequenced the gut microbiomes of both groups and measured a battery of biological markers associated with frailty — the progressive loss of muscle, strength, and metabolic resilience that defines healthy aging from unhealthy aging.

The result was that the participants who had shifted towards the Mediterranean pattern showed microbiome changes — increases in specific taxa including *Faecalibacterium prausnitzii*, *Roseburia*, and other SCFA producers; decreases in taxa associated with inflammation — that correlated, across the entire cohort, with reduced frailty markers and improved cognitive function. The changes were modest but real. More importantly, they were mediated by the microbiome: a statistical analysis showed that the effect of the diet on frailty was partly explained by the effect of the diet on specific microbial taxa and their metabolic outputs. This is what clinical epidemiologists call “mediation analysis,” and it is one of the cleanest ways to show that a mechanism is doing real work in a real-world intervention.

NU-AGE did not show a revolution. Elderly people put on a Mediterranean diet for a year did not suddenly become young. But they became measurably less frail than the controls, and the fingerprint of the microbiome change was visible in the data. For a dietary trial at this scale, in this age group, the result is unusual in its clarity.

Meslier: the shorter trial in a younger group

A second 2020 study, by Vanessa Meslier and colleagues working in Paris and Naples, asked a different question: in overweight and obese adults, does a Mediterranean diet intervention produce microbiome and metabolic changes independent of weight loss? [REF:meslier2020]

The study randomised 82 overweight or obese subjects to an 8-week isocaloric Mediterranean diet intervention or a habitual diet control. “Isocaloric” means the participants were not asked to eat less; they were asked to change *what* they ate without changing *how much*. This is an unusual design. Most diet trials bundle compositional change with caloric restriction, and it becomes impossible to tell whether the benefits come from the food composition or from simply eating fewer calories.

After eight weeks, the Mediterranean group had significant reductions in plasma cholesterol — roughly 10 milligrams per decilitre — with no change in body weight. The gut microbiome had shifted towards a more fibre-degrading, SCFA-producing composition. Plasma and faecal metabolomic profiles showed changes consistent with increased polyphenol and fibre intake. Because

weight had not changed, the benefits had to be coming from the change in what the subjects were eating rather than from weight loss.

This is an important finding because weight loss is the usual confounder in diet trials. Many dietary patterns produce temporary benefits that are actually benefits of transient caloric restriction, and the benefits vanish when weight is regained. The Meslier study carves out a cleaner signal: a diet change produced microbiome and metabolic improvements *without* a weight change, suggesting that the Mediterranean pattern has effects that are independent of its role in weight management. You do not have to lose weight to benefit from eating this way.

The pattern is a delivery system

Why does the Mediterranean pattern work so well as a microbiome intervention?

Because, under its Italian and Greek and Lebanese clothing, it is a delivery system for everything this chapter has so far argued matters. The fibre content is high, because it is a predominantly plant-based pattern with legumes, whole grains, vegetables, fruits, and nuts in almost every meal. The plant species count is high, because Mediterranean cuisines are built around herbs, spices, and mixed-vegetable preparations. The polyphenol content is high — we will come back to why in section 20.7 — because olive oil, red wine in moderation, coffee, tea, dark greens, and herbs are polyphenol powerhouses. The fermented-food content is meaningful, because the traditional Mediterranean diet includes yogurt, kefir, aged cheeses, olives, and in some regions sourdough breads. The saturated fat content is modest. The ultra-processed content is near zero in the traditional form, because the pattern predates the industrial food system that defines Western ultra-processed eating.

If you were designing a diet from first principles to feed a diverse, functional, SCFA-producing gut microbiome while also keeping the polyphenol and fermented-food contributions high, you would arrive somewhere very close to the Mediterranean pattern without ever having heard of it. It is the rare case in nutritional science where traditional wisdom and contemporary mechanism align so clearly that they seem to have been designed together.

One caveat is important. “Mediterranean diet” as sold in supermarkets, restaurants, and some commercial meal-kit services is often a costume rather than a substance. A “Mediterranean salad” drenched in commercial dressing, accompanied by white bread and a sugar-sweetened drink, is not the Mediterranean diet of Crete in 1960. The evidence base comes from the traditional pattern: mostly plants, in variety, with olive oil as the dominant fat, with small amounts of fish and even smaller amounts of red meat, with very little processed food, and with meals eaten slowly in social company. When this chapter recommends “a Mediterranean-style pattern,” it means the real thing, not the marketing label.

The last point — meals eaten slowly in social company — is not just cultural decoration. We will return to it in section 20.10, when we look at *when* you eat rather than *what* you eat. The Mediterranean pattern is not only a composition. It is also, in its traditional form, a rhythm. Both matter.

20.6 Fermented Foods: The Other Living Diet

A global habit, almost forgotten

Before refrigeration, almost every human culture preserved food by letting selected microbes grow on it. Milk became yogurt, kefir, and cheese. Cabbage became sauerkraut, kimchi, and a dozen regional relatives. Soy became miso, tempeh, natto, and soy sauce. Fish became the sauces of Southeast Asia and the garum of the Romans. Grains became sourdough bread and the starters behind every traditional beer. Tea became kombucha. Cassava became fufu. Somewhere between five and ten thousand years ago, humans learned that if you let the right microbes take over a food, the food would not only keep but also change — becoming more digestible, more flavourful, more nutritious, and, in many cases, better tolerated by people who could not drink plain milk or eat plain soy or chew plain cabbage leaf. Fermented foods, in this sense, are one of the oldest technologies in human history [REF:marco2017].

Then, over the course of the twentieth century, most of them quietly slipped off the Western plate. Refrigeration removed the practical necessity. Industrial production replaced live-culture yogurt with pasteurised “yogurt-style” desserts. Sauerkraut in most supermarkets is now pasteurised and shelf-stable — killed before it gets to you. Sourdough became the niche product of artisan bakeries while commercial bread switched to fast-acting baker’s yeast. The food tradition of microbial cooperation, maintained for millennia, thinned to a few pockets: natto in Japan, kimchi in Korea, kefir in parts of Eastern Europe, lambic beers in Belgium, and live yogurt in those dwindling corners where the word still means what it used to mean.

This matters for the microbiome in ways we have only recently begun to understand, and the evidence that has accumulated in the last few years is, in my view, the single most surprising development in the dietary microbiome literature. Not the fibre story — we knew fibre was important, and the details are refinements. The fermented-food story is closer to a discovery, and it came from one of the best-designed dietary trials the microbiome field has yet produced.

The Stanford trial

In 2021, a team led by Hannah Wastyk, Erica Sonnenburg, Justin Sonnenburg, and Christopher Gardner at Stanford published a paper in *Cell* that is, I think, a candidate for the most important dietary microbiome trial of the decade [REF:wastyk2021].

The design was clean in a way that trials of this kind rarely are. Thirty-six healthy adults were randomised into two arms. One arm was asked to progressively increase their fermented food intake over four weeks, working up from a baseline of near zero to a target of six servings of fermented foods per day, and then to hold that level for another six weeks — ten weeks total. The other arm was asked to progressively increase their fibre intake over the same period, working from a baseline of around 22 grams per day up to about 45 grams per day, and hold. Both interventions are, on paper, things we would expect to improve the microbiome, and the research team’s hypothesis was that both arms would show benefits, with the fibre arm probably doing more.

The team took stool samples, blood samples, and peripheral blood mononuclear cells at baseline, during the intervention, and after washout. They sequenced the microbiome in depth. They measured 93 circulating inflammatory markers. They profiled cytokine production by the participants’ own immune cells after ex vivo stimulation — a way of asking not just “are the inflammatory markers high?” but “how inflammatory is the immune system if you poke it?” In short, they measured not only the ecological change but also the physiological consequence.

Here is what they found, and it is worth slowing down to take it in.

The fermented-food arm showed a clear and significant increase in gut microbial diversity over the ten weeks. This is one of the few dietary interventions that has ever produced a measurable diversity increase in a randomised human trial. The fibre arm, at the same time, did *not* show an increase in diversity. The fibre arm did show other changes — shifts in the expression of microbial carbohydrate-degrading enzymes, among others — but the diversity needle did not move.

The immunology was even more striking. Nineteen of the 93 inflammatory markers the team measured went *down* significantly in the fermented-food arm. Markers associated with chronic low-grade inflammation — the kind implicated in metabolic disease, cardiovascular disease, and age-related frailty — dropped across the board. The immune cells of participants in the fermented-food arm were less reactive when stimulated, suggesting a reduction in baseline inflammatory tone. The fibre arm showed no such reduction. On the immune outcomes the trial was designed to detect, the fermented-food arm did what the researchers had predicted the fibre arm would do, and the fibre arm did not.

There are caveats. The trial was small — eighteen people per arm. It was only ten weeks. Both interventions are known to be beneficial, so the comparison is not “good versus bad” but “good versus very good.” And the finding that high-fibre intervention did not reduce inflammation in this trial contradicts earlier evidence from other trials, which forces us to be careful about generalising. One interpretation is that fibre needs longer than ten weeks to show its anti-inflammatory effect, particularly if the participant’s microbiome has been depleted by years of low-fibre eating and no longer contains the species that would do the fermenting. The Stanford team hinted at this: they pointed out that many of their fibre-arm participants showed evidence that their microbes simply could not handle the increased substrate, producing inflammatory fermentation byproducts rather than the expected shift to butyrate production. The machinery was not there. You can shovel oats into a gut whose fibre-fermenting species are missing, but the oats will not turn into butyrate. They will mostly just leave.

That interpretation is important for the practical section of the chapter, because it suggests that fermented foods may have a particular role for people whose microbiomes have been *depleted* — whose fibre-fermenting machinery is partly missing. Fermented foods deliver not only substrate but also live microbes, and at least some of those microbes, or their metabolic products, appear to do useful work.

What fermented foods actually do

The Stanford trial does not, on its own, tell us *how* fermented foods produce their effect. But we have a reasonable working picture, and it involves at least three mechanisms.

First, live microbes reach the distal gut. Not many of them. Not permanently. Most of the bacteria in a spoonful of yogurt are destroyed by stomach acid or outcompeted by the residents. But some arrive, some interact with the resident community, and some — particularly spore-formers and acid-tolerant species — persist long enough to do something useful. Taylor and colleagues, in a 2020 observational study, showed that habitual fermented-food consumers carry distinct microbial and metabolomic signatures compared with non-consumers, suggesting that the dietary habit leaves a measurable trace in the gut [REF:taylor2020].

Second, fermented foods deliver *microbial metabolites* that were produced during fermentation itself — short-chain peptides, bioactive amines, vitamins, exopolysaccharides, and compounds spe-

cific to the fermenting organism. When a yogurt culture ferments milk, it does not just thicken it; it enzymatically pre-digests some of the proteins, generates bioactive peptides from casein, produces lactic acid that alters the mineral availability of the product, and enriches the food with vitamins and precursors that the starting milk did not contain. You are not just eating dairy. You are eating the collected output of a microbial factory that worked on your behalf before you opened the container.

Third, fermentation *removes* components that would otherwise be problematic. Sourdough fermentation partially degrades the FODMAPs in wheat — the fermentable oligosaccharides that trigger bloating in many people — which is one reason that some individuals who feel bad after modern bread can tolerate traditional long-fermented sourdough without trouble. Kimchi and sauerkraut degrade some of the raw cabbage glucosinolates that cause gas. Tempeh and miso partially degrade phytates in soy, improving mineral bioavailability. Fermentation, in each case, is a kind of pre-processing that shifts the food from “raw plant” to “microbially prepared plant,” and the prepared version is often friendlier to the eater.

Getting six servings a day

Six servings a day sounds intimidating, but a serving, in the Stanford trial, was modest: a quarter-cup of kimchi or sauerkraut, half a cup of kefir or kombucha, a few ounces of yogurt, one or two pieces of aged cheese. The participants did not replace their normal meals with barrels of pickles. They added small amounts of fermented foods across the day: a dollop of yogurt at breakfast, a splash of kombucha mid-morning, a forkful of sauerkraut on the lunch plate, a few slices of a fermented cheese in the afternoon, a small portion of kimchi with dinner, a cup of kefir before bed. Six servings became achievable once the definition was right.

The practical advice, tiered by strength of evidence and by ease, comes out as follows. *If you do nothing else from this section, eat one serving of a real fermented food at most meals.* Yogurt with live cultures, kefir, traditional sauerkraut (refrigerated, not pasteurised, with visible bubbles or the note “contains live cultures” on the label), traditional kimchi, aged cheeses (which contain surviving microbial communities even though they are not typically described as fermented foods by lay readers), miso paste (added to soups after the soup is removed from heat to preserve the live cultures), tempeh, natto if you can find it and stomach it, kombucha (bearing in mind the sugar content of sweeter commercial varieties), and long-fermented sourdough bread from a bakery that actually ferments overnight rather than using commercial starter for flavour alone.

Avoid the supermarket impostors. “Pickles” produced by vinegar brining are not fermented — the vinegar is doing the preserving, not the microbes. “Yogurt” that lists “milk, cream, fruit, sugar, gelatin, pectin, natural flavours” without mentioning live cultures is almost certainly pasteurised after fermentation and contains no live organisms. Pasteurised sauerkraut from the canned-goods aisle is, biologically, cooked cabbage. Check the refrigerator section; read labels; look for visible bubbles or sediment; if in doubt, make your own. A batch of sauerkraut from one cabbage, one tablespoon of salt, and a jar takes about fifteen minutes to start and two weeks to finish. The resulting product is more expensive per jar than anything in the supermarket, because your time is valuable, but for roughly the cost of a cabbage you can build a supply of the real thing for a month.

The caveat paragraph

Before we move on, the obligatory caveat. Fermented foods are not a treatment for disease. The Wastyk trial was in healthy adults, and the outcomes were inflammatory markers rather than clin-

ical events. People with severely compromised immune systems — chemotherapy patients, transplant recipients, people on high-dose immunosuppressants — should talk to their clinicians before starting high-level fermented-food intake, because some live cultures can, rarely, cause problems in profoundly immunosuppressed hosts. People with histamine intolerance may find that aged cheeses and some fermented foods worsen their symptoms because fermentation produces biogenic amines. People on medications such as MAO inhibitors need to watch their tyramine intake, which is elevated in some aged cheeses and fermented soy products. These are real considerations for real people. The general population advice — most readers of this chapter — is that adding real fermented foods to the diet is one of the best-supported, lowest-risk, and most immediately effective things the microbiome literature currently points to.

20.7 Polyphenols: Why It Depends Whose Garden You Have

The chemistry of plant defence

Plants, unlike animals, cannot run away from what threatens them. They cannot dodge the insect that wants to chew them, the fungus that wants to rot them, or the sun that wants to oxidise their leaves. Instead, they fight chemically. Over four hundred million years of evolution, land plants have developed an extraordinary arsenal of secondary metabolites — molecules not directly involved in growth and reproduction, but deployed to deter enemies, attract allies, or protect against environmental stress. Many of these secondary metabolites are bitter, astringent, and vividly coloured, which is why the most chemically defended parts of plants often taste the most interesting and photograph the best.

A subset of these chemicals, called polyphenols, are defined by their structure: they carry multiple phenolic rings, chemical groups that can donate electrons and thereby neutralise reactive molecules. There are somewhere between eight and ten thousand known polyphenols, clustered into families: flavonoids (including the catechins of tea, the anthocyanins of berries, and the flavones of parsley and celery), phenolic acids (including the chlorogenic acid of coffee), stilbenes (including the resveratrol of red wine), lignans (in flaxseed and whole grains), and tannins (in wine, tea, and many fruit skins). Each family has its distinctive chemistry. What they share is that when you eat them, something surprising happens: most of them are not absorbed.

Estimates vary, but somewhere between 90 and 95 percent of dietary polyphenols pass through the small intestine and reach the colon intact or partly altered. They are too large to be readily absorbed across the intestinal wall, or too tightly bound to the plant's fibre matrix, or simply not in a form the enterocytes can transport. This fact used to be considered a problem — how can a compound that barely gets into the blood matter much for health? — until it became clear that the polyphenols are not meant for the enterocytes in the first place. They are meant for the microbes. And what the microbes do with them is where the story gets interesting [REF:cortes-martin2020].

Urolithin: the pomegranate story

Consider ellagitannins, a class of polyphenol concentrated in pomegranates, walnuts, raspberries, strawberries, and certain teas. When you eat a pomegranate, the ellagitannins arrive in your colon largely intact. There, some gut microbes — notably species in the *Gordonibacter* and *Ellagibacter* genera — possess the enzymes to break them down, releasing a smaller molecule called ellagic acid. Some microbes then process the ellagic acid further, through several intermediate steps, to produce

a compound called urolithin A. Urolithin A, unlike its parent molecules, is small enough and hydrophobic enough to be absorbed across the colonic wall. It enters the circulation and reaches other tissues. In cell and animal experiments, urolithin A has been shown to have effects on mitochondrial health, autophagy, and muscle function. Human trials, while early, suggest it may contribute to the benefits of polyphenol-rich diets in ageing-related muscle decline.

Here is the catch, and it is a big one. Not everybody makes urolithin A. Studies across multiple populations have found that people fall into three broad groups, which the research team of Francisco Tomás-Barberán in Murcia have called “metabotypes.” Metabotype A people convert ellagitannins efficiently into urolithin A. Metabotype B people produce mostly a different metabolite called urolithin B, which has weaker biological activity. Metabotype O people — and this is somewhere between 10 and 40 percent of the population, depending on the study — produce almost no urolithin at all. They simply do not carry the microbes that run the conversion, and their ellagitannins pass through them without yielding the useful downstream product [REF:tomas-barberan2017].

This means that the same handful of walnuts, eaten by three different people, produces three different systemic exposures to urolithin. For one person, the walnut is a delivery device for a potentially beneficial small molecule. For another, it delivers only the raw ingredient and the weaker derivative. For a third, it delivers nothing of the kind. The walnut is not different. The people are.

The equol story, and others

A similar pattern applies to soy isoflavones. Soy contains daidzein, one of the best-studied phytoestrogens. In some people, gut microbes convert daidzein into a molecule called equol, which has stronger oestrogenic activity than daidzein itself and appears to be the compound responsible for many of the effects attributed to soy — including, possibly, the reduced incidence of certain hormone-sensitive cancers in populations that eat soy from childhood. Only some people are equol producers. In Western populations, the proportion is often below 30 percent. In traditional Asian populations, the proportion tends to be higher, partly for genetic reasons but mostly, we think, because early-life dietary exposure and the associated microbial community favour the necessary converting species. If you are not an equol producer, the benefits attributed to soy in the epidemiological literature may not accrue to you in the same way.

The same principle applies, to varying degrees, to many other polyphenol classes. Flavan-3-ols from cocoa and green tea are processed by the microbes into phenyl-γ-valerolactones and other absorbable small molecules. Lignans from flaxseed are converted to enterolignans like enterodiols and enterolactone. Anthocyanins from berries are modified into a whole cascade of smaller phenolic metabolites. In each case, the ultimate systemic exposure to the biologically active product depends on whether the eater’s microbiome contains the necessary converting species, and the answer is frequently “partly” or “not much.”

What this means for you on Tuesday

It would be tempting to take this as a reason to give up on polyphenol-rich foods. After all, if you are not going to get the urolithin, why bother with the walnut? The answer is that the parent compounds, even without microbial conversion, carry useful effects of their own — direct antioxidant activity in the gut lumen, modification of the microbial community itself (several polyphenols preferentially feed beneficial taxa and suppress certain pathogens), and bioactivity from the smaller fraction that is absorbed intact. More importantly, the whole-food sources of polyphenols — berries, coloured vegetables, tea, coffee, dark chocolate, olives, olive oil, herbs and spices, red wine in

moderation, whole grains — are also, almost without exception, sources of fibre, vitamins, minerals, and plant diversity. Even if you are a metabotype O, a diet rich in polyphenolic foods is also a diet rich in everything else good.

The more sophisticated reading of the metabotype story is that it explains why population-level studies of polyphenol intake and disease sometimes produce inconsistent results. If the benefit depends on having a particular microbiome, then the average effect across a heterogeneous population will look smaller than the effect in the subset who can actually produce the active metabolite. This is a recurring theme in diet and microbiome research: the average hides the individuals, and some of the “inconsistencies” that have made nutritional epidemiology so frustrating over the decades may turn out to reflect real differences in who processes what.

The practical rule is simple. Eat polyphenol-rich foods in variety. Berries (fresh or frozen — frozen are often higher in polyphenols because they were picked ripe and preserved quickly). Tea, especially green tea. Coffee if you enjoy it. Dark chocolate (70% or higher, in small amounts, not as a substitute for actual food). Extra-virgin olive oil, which carries hydroxytyrosol and oleocanthal as well as fat. Olives. Herbs and spices — every culture’s traditional cuisine is a polyphenol delivery device disguised as flavouring. Red wine if it is part of your life, in modest amounts, with food. Nuts, particularly walnuts. Whole grains. Legumes, especially those with coloured seed coats. Do not be disappointed when headlines claim that this week’s superfood is “the answer”; there is no answer, only a rich and varied plate, and the microbes in your own particular gut will make of it what they can.

If, in five or ten years, commercial microbiome testing becomes cheap and reliable enough to tell you which polyphenol converters you carry, that information may become actionable. Today, it is not. Assume you are probably a partial converter for most classes, eat the foods anyway, and let your own particular ecosystem do its best.

20.8 The Other Side of the Plate: Protein, Fat, and the Metabolites You Don’t Want

TMAO: when your microbes turn dinner into a risk factor

In 2011, a group led by Stanley Hazen at the Cleveland Clinic published a paper in *Nature* that did something rare in cardiology: it added a new player to the list of molecules that predict heart disease, and the new player was not one of the usual suspects [REF:wang2011]. The molecule was trimethylamine-N-oxide, or TMAO, a small compound that had been known to chemists for a long time but had never been seriously considered as a cardiovascular risk factor until Hazen’s group showed that elevated blood levels of TMAO were strongly and independently predictive of heart attack, stroke, and cardiovascular death — even after adjusting for every traditional risk factor they could think of.

Where does TMAO come from? From your bacteria. Specifically, certain gut microbes break down dietary choline — abundant in eggs, red meat, poultry, and fish — into a gas called trimethylamine, or TMA. The TMA is absorbed into the portal vein, travels to the liver, and is oxidised by a host enzyme (FMO3) into TMAO, which then enters the systemic circulation. The TMAO is what seems to promote atherosclerosis, apparently by altering cholesterol handling in macrophages and contributing to foam-cell formation in the arterial wall.

In 2013, the Hazen group published a second paper, this one focused on a different precursor: L-carnitine, a molecule found in high concentrations in red meat [REF:koeth2013]. The mechanism was similar. Gut microbes convert carnitine to TMA; the liver oxidises it to TMAO; the TMAO contributes to atherosclerosis in mouse models and correlates with cardiovascular events in humans.

Here is the part of the Koeth study that made people sit up and pay attention. The researchers fed a steak (or, in the formal experiment, a standardised serving of beef) to a group of omnivores and measured their blood TMAO response. Levels rose. They then fed the same carnitine challenge to a group of long-term vegans and vegetarians. Levels barely moved. The vegans and vegetarians did not make TMAO from dietary carnitine, because they did not carry the bacterial populations that perform the conversion. Long-term avoidance of meat had, in effect, de-trained the microbial community for this particular substrate. Give it meat, and nothing happened. Give the omnivore meat, and the bacterial machinery that had been sustained by decades of meat-eating went to work.

This finding complicates the usual narrative about red meat and cardiovascular disease. The traditional interpretation — that meat is bad because of its saturated fat content — is partly true but incomplete. The Hazen-Koeth line of work suggests that a significant portion of the harm attributed to red meat is actually mediated by the microbial response to it, and that the microbial response itself depends on who you are and what you have been eating. The steak is not the only variable. The population of bacteria in your colon, shaped by years of previous dinners, is another.

It also suggests a form of partial reversibility. If you drop meat from your diet for long enough, the TMAO-producing populations shrink and the conversion slows. If you then eat meat occasionally, the conversion is partial. You do not have to become a lifelong vegan to benefit from reduced TMAO exposure; you may simply have to make meat something other than the centre of most meals. This is, again, what the Mediterranean pattern has been quietly telling us for sixty years: a small portion of fish or meat, surrounded by a large portion of plants, eaten a few times a week rather than most days, looks very different, in microbial terms, from a diet built around meat as the main event.

Saturated fat, taurine-bile, and the wadsworthia story

The second protein-and-fat story is more surprising, and it comes from a 2012 paper by Suzanne Devkota working in Eugene Chang's laboratory at Chicago [REF:devkota2012]. Devkota's study was in mice, but the mechanism it described is, as far as we can tell, fully applicable to the human bowel.

The experimental setup was this. Mice genetically engineered to be susceptible to inflammatory bowel disease (IL-10 knockout mice, missing a key anti-inflammatory signal) were placed on one of three diets: low fat, high fat with the fat coming from lard (mostly saturated), or high fat with the fat coming from safflower oil (mostly polyunsaturated). The team then measured the composition of the gut microbiota and the development of colitis.

On the low-fat diet, the susceptible mice mostly stayed healthy. On the polyunsaturated high-fat diet, they also mostly stayed healthy — so the problem was not fat per se. But on the saturated-fat diet, something striking happened. The composition of the mouse bile acid pool shifted. Normally mouse bile contains a mixture of glycine-conjugated and taurine-conjugated bile acids. On the high-saturated-fat diet, the taurine-conjugated fraction surged, because dealing with the higher fat load required more bile, and the taurine conjugates were preferentially produced. This was a subtle biochemical shift at the level of bile composition.

It was then amplified by the microbes. One bacterial species in particular, *Bilophila wadsworthia*,

thrives on taurine-derived sulphur. When the taurine-conjugated bile acids increased, *Bilophila wadsworthia* bloomed from a minor to a major component of the microbiota. Its metabolism produced hydrogen sulphide, a pro-inflammatory gas. The inflammatory burden on the colonic wall increased. And the genetically susceptible mice, in whom any additional inflammatory trigger could push the gut into overt disease, developed colitis.

The study is in mice with a specific genetic vulnerability, and we should not extrapolate it to every person who eats a cheeseburger. But the mechanism — saturated fat drives a bile acid shift that expands a sulphur-loving pathobiont that produces an inflammatory gas — is documented, biochemically coherent, and at least partially replicable in human studies that have since followed. *Bilophila wadsworthia* is present in the human gut and does expand on high-saturated-fat diets. Its sulphide production is real. Whether this produces frank colitis in most people is unclear; whether it contributes to the lower-grade inflammation associated with high-saturated-fat eating is plausible.

The speed of change

One more study in this cluster deserves a mention, because it tells us how fast dietary changes show up in the microbiome. In 2014, a team led by Lawrence David, then at Harvard, published a paper called “Diet rapidly and reproducibly alters the human gut microbiome” [REF:david2014]. The design was elegant. Ten healthy adults were fed, for five consecutive days, one of two extreme diets. The “plant-based” diet was essentially a vegan diet with high fibre and high complex carbohydrates. The “animal-based” diet contained meat, eggs, and cheeses at every meal, with almost no plant foods at all. Stool samples were collected before, during, and after each intervention.

Within 24 to 48 hours of starting either diet, the microbiome composition had shifted detectably. Within four days, the shifts were large. The animal-based diet promoted bile-tolerant organisms (*Bilophila*, among others) and suppressed Firmicutes that metabolise plant polysaccharides. The plant-based diet did the opposite. When the participants returned to their habitual diets, the changes reversed over a few days.

The take-home is both encouraging and sobering. Encouraging: your microbiome responds fast. A week of eating better is already starting to show results in the microbial community. Sobering: a week of eating badly has a similar speed of effect in the wrong direction, and if “a week of eating badly” becomes “a year of eating badly,” the cumulative shift can be substantial and, as we saw in the Sonnenburg extinction experiments, not fully reversible.

Enterotypes and the long term

There is one more layer to the picture, introduced by Gary Wu and collaborators at the University of Pennsylvania in 2011 [REF:wu2011]. They asked whether long-term dietary patterns — not what someone ate last week but what they had eaten for years — could be read off the gut microbiome. Their answer was yes, and they proposed a framework called “enterotypes”: broad categories of microbiome composition that cluster around different dominant genera.

The simplest version of the enterotype story, and the one that has survived the most scrutiny, is a spectrum between two extremes. At one end, people with a *Bacteroides*-dominated microbiome tend to have had long-term diets high in animal protein and animal fat. At the other end, people with a *Prevotella*-dominated microbiome tend to have had long-term diets high in fibre and plant-based carbohydrates. Short-term dietary change, as David showed, produces fast but partial shifts. Long-term dietary pattern produces deeper shifts that, in effect, select which ecosystem you carry.

Taken together, this cluster of studies — Hazen, Koeth, Devkota, David, Wu — tells a coherent story. What you eat today has fast effects on your gut microbes. What you eat across years shapes which microbes you carry, which metabolites they can make from your food, and how those metabolites act on the rest of your body. Animal foods are not the enemy. But a diet in which animal foods dominate over a long period trains a microbial community that produces more of the metabolites we don't want (TMAO, sulphides, secondary bile acids in inflammatory configurations) and less of the metabolites we do (butyrate and its SCFA cousins). The traditional Mediterranean pattern's recommendation of "meat a few times a week, plants at every meal" is, in hindsight, the right balance not because plants are virtuous and meat is sinful, but because this balance trains a microbial ecosystem whose metabolic outputs favour the host.

20.9 Ultra-Processed Food: When the Package Is the Problem

A category that didn't exist a generation ago

Walk through a modern supermarket and ask yourself a simple question: which of the items on these shelves would have been recognisable to your great-grandmother? The fruit and vegetable aisle, yes. The butcher and fish counter, mostly. The dry-goods aisle — flour, rice, dried beans — mostly. The dairy cabinet, with some caveats. Everything else — the coloured boxes, the plastic pouches, the brightly labelled tubs, the frozen ready meals, the "protein" bars, the "healthy" snacks, the "natural" beverages — contains products that did not exist in their current form before about 1970 and, for the most part, did not become a major fraction of the Western food supply until the 1980s and 1990s.

In 2009, a Brazilian epidemiologist named Carlos Monteiro proposed a classification system for foods based not on their nutrient content but on how much they had been industrially transformed. He called it the NOVA system. It has four categories. Group 1 is *unprocessed or minimally processed foods* — fruits, vegetables, grains, legumes, nuts, eggs, milk, meat, fish, spices, herbs. Group 2 is *processed culinary ingredients* — oils, butter, vinegar, salt, sugar, flour. Group 3 is *processed foods* — foods made by combining Group 1 and Group 2 items in recognisable ways, including traditional bread, cheese, canned vegetables, and cured meats. Group 4 is *ultra-processed foods*, and the definition is worth quoting, because the category is not simply "things with a lot of ingredients." Ultra-processed foods are, in Monteiro's formulation, industrial formulations that typically contain substances not found in home kitchens — protein isolates, modified starches, hydrogenated oils, high-fructose corn syrup, emulsifiers, stabilisers, artificial flavours, artificial colours, non-nutritive sweeteners — and that are designed to be hyper-palatable, shelf-stable, and profitable.

By this classification, ultra-processed foods now account for somewhere between 50 and 60 percent of total energy intake in the United States and the United Kingdom, and a substantial and rising fraction in most other industrialised countries. This is a dietary category that has moved, within a generation, from non-existent to dominant. And the evidence is accumulating, from multiple directions, that the microbiome is part of why it matters [REF:zinocker2018].

Emulsifiers: the wallpaper dissolver

Consider a product as innocent-sounding as commercial ice cream. Check the label. You will almost certainly find, somewhere in the ingredient list, one or more of: carboxymethylcellulose (also written CMC or E466), polysorbate 80 (E433), mono- and diglycerides, carrageenan, lecithin, or

xanthan gum. These are emulsifiers and stabilisers. Their job is to keep water and fat mixed smoothly, prevent ice crystals from forming, and give the product its characteristic mouthfeel. They are in ice cream, but they are also in commercial bread, salad dressings, plant milks, processed cheese, margarine, ready meals, protein shakes, and a substantial fraction of the processed food aisle. Most of them are regarded as safe in the sense that they do not cause acute toxicity at the doses used.

In 2015, Benoit Chassaing and Andrew Gewirtz at Georgia State published a paper in *Nature* that added a worrying footnote to that long-standing conclusion [REF:chassaing2015]. They fed mice CMC and polysorbate 80 at doses comparable to ordinary human exposure through the processed food supply — not experimental overdoses, but the kind of intake a person would get from a Western diet. They then measured microbiota composition, mucus layer integrity, inflammatory markers, and body weight.

The findings were, in a word, unsettling. The emulsifier-fed mice showed a thinning of the mucus layer — the same mucus layer we discussed in the fibre section, the layer that is supposed to separate the bacteria in the lumen from the epithelial cells of the gut wall. The bacteria, instead of living at a respectful distance, were now coming closer to the epithelium. Chassaing and Gewirtz called this “microbiota encroachment.” The microbiome composition shifted towards pro-inflammatory configurations. The mice developed low-grade inflammation, insulin resistance, and features of metabolic syndrome. In mice genetically predisposed to inflammatory bowel disease, frank colitis developed.

The reasonable scientific response was “this is in mice; do we see it in humans?” In 2022, Chassaing and colleagues published the answer: a randomised, controlled, double-blind human feeding trial in which healthy adults were given diets containing CMC at levels consistent with typical dietary exposure, or a matched control diet without CMC, for eleven days [REF:chassaing2022]. The sample was small — sixteen participants — but the experimental control was tight, and the outcomes were measured carefully.

The results confirmed, in humans, the direction of the mouse findings. Participants on the CMC diet showed reduced microbial diversity, alterations in the faecal metabolome, and — crucially — evidence of microbiota encroachment on the mucus layer in a subset of participants. Eleven days of an emulsifier that had been classified as safe for decades produced measurable changes in the way the gut microbes related to the gut wall.

This does not mean CMC is acutely dangerous. It does mean that a food additive which reaches us in small quantities through many products, day after day, year after year, is not as biologically inert as the regulatory history had assumed. And the list of emulsifiers that the Chassaing trial tested is only two molecules out of dozens that are routinely used in the ultra-processed food supply. We do not know, yet, what each of the others does. Polysorbate 80 behaves similarly to CMC in animal studies. Carrageenan has a longer-standing literature associating it with intestinal inflammation. Others have not been adequately studied.

The reasonable position, pending further data, is that dietary emulsifiers at Western food-supply levels are now a plausible contributor to the low-grade inflammation that characterises so much of contemporary chronic disease, and that reducing exposure to them is a sensible precaution even in the absence of definitive long-term outcome data. Practically, this means reading ingredient lists, favouring foods with short ingredient lists that contain only things you could buy at a market, and accepting that the creamy-mouthfeel ice cream of the supermarket freezer is not as trivial a treat as it looks on the label.

Non-nutritive sweeteners: the diet soda surprise

A parallel story, equally counterintuitive, has emerged around non-nutritive sweeteners. These are the compounds — saccharin, aspartame, sucralose, stevia glycosides, acesulfame potassium — that provide sweetness with few or no calories, and that have been marketed for decades as the healthy alternative to sugar. They were, for most of their history, considered microbiologically irrelevant on the theory that a compound which is not metabolised by the human body is also not metabolised by the gut microbiota.

In 2014, a group led by Eran Elinav and Eran Segal at the Weizmann Institute in Israel published a paper in *Nature* that turned this assumption upside down [REF:suez2014]. They showed that saccharin fed to mice at doses corresponding to the FDA's acceptable daily intake caused glucose intolerance — the precursor state to type 2 diabetes. They then showed that the mechanism was microbial: the saccharin altered the gut microbiota composition, and when faecal microbiota from saccharin-fed mice were transplanted into germ-free mice, the recipients developed glucose intolerance too. The microbial shift was the mediator. In a small pilot experiment in humans, a subset of volunteers who consumed saccharin for a week showed similar patterns — altered microbiota and impaired glucose responses — while another subset did not respond.

The 2014 paper was important but small and its human arm was limited. The definitive follow-up came in 2022, again from Suez, Elinav, and Segal, and was published in *Cell* [REF:suez2022]. This was a properly powered, double-blind, placebo-controlled human trial. One hundred and twenty healthy adults who had not been consuming non-nutritive sweeteners were randomised into six groups: saccharin, sucralose, aspartame, stevia, a placebo (glucose at a matched dose), or no supplement. The test sweeteners were given at doses well below the FDA's acceptable daily intakes. Outcomes included microbiome composition, metabolomic profiles, and glycemic response to oral glucose challenges.

The findings, taken carefully, are these. All four sweeteners altered the gut microbiome and the plasma metabolome, albeit in different directions. Two of them — saccharin and sucralose — also produced measurable impairment of glycemic responses in the participants who received them, with the impairment varying strongly between individuals. When faecal microbiota from the most responsive human participants were transplanted into germ-free mice, the mice recapitulated the glucose-intolerance phenotype — proof that the microbial changes were sufficient, on their own, to produce the glycemic effect.

Non-nutritive sweeteners, in other words, are not microbiologically neutral. They interact with the microbiome, the microbiome interacts with the host, and in some individuals the consequence is worse rather than better glucose handling. The person who drinks a diet cola in place of a sugar cola is not, on this evidence, getting the metabolic neutrality the marketing promises. Some people may be, some people are not, and we cannot currently tell in advance which category any given reader falls into.

The practical position for the general reader is cautious. If you are currently drinking multiple artificially sweetened beverages per day, it is worth considering whether the category is doing you the metabolic favour you had assumed. Reducing or eliminating them, substituting water, unsweetened tea and coffee, or a small amount of sugar, is a reasonable move. The same applies, more broadly, to any ultra-processed product whose “diet” or “light” credentials rest on sweetener substitution.

The Western diet as an ecological insult

The Zinöcker and Lindseth 2018 review pulled these strands — fibre depletion, emulsifier exposure, sweetener exposure, food-matrix disruption — into a single framework [REF:zinocker2018]. Their argument, in essence, is that the Western ultra-processed food system is best understood not as a specific nutrient problem but as a systemic ecological insult to the gut microbiome. Individual ingredients do individual damage. The combination — low MAC, constant emulsifier exposure, intermittent sweetener exposure, disrupted food matrices, displaced fermented foods, displaced plant diversity — acts on the ecosystem in multiple overlapping ways at the same time.

This is why single-nutrient approaches to improving dietary health — “eat less fat,” “eat more fibre,” “avoid sugar” — have historically produced modest and inconsistent results. The problem is not any one nutrient. The problem is the architecture. A diet built around ultra-processed products will be nutritionally inadequate in many overlapping ways, and tinkering with one nutrient while leaving the architecture intact produces small and often transient benefits. The alternative is not another single-nutrient rule. It is a structural shift: making the base of your diet minimally processed foods — Monteiro’s Groups 1, 2, and 3 — and letting Group 4 be a minor and occasional category rather than a dominant one.

I am aware that this sounds, in 2026, either obvious or impossible. Obvious, because it is what every sensible dietary writer for thirty years has been saying in different words. Impossible, because the convenient, affordable, and socially normal foods in most Western cities are predominantly ultra-processed, and asking a working family to “just cook from scratch” without acknowledging the time, money, and skill barriers that task involves is glib. I am not going to pretend those barriers do not exist. What I will say is that any move in the direction of minimally processed foods — even a partial one — is likely to pay returns, and that the returns will be disproportionately larger than equivalently sized changes within the ultra-processed category. Switching from one brand of packaged breakfast cereal to another does little. Replacing the breakfast cereal with oats and a handful of nuts does a lot. The difference is structural.

20.10 When You Eat: The Circadian Microbiome

The clock inside the clock

Until about a decade ago, most of us thought of the gut microbiome as a community that sat in the colon doing roughly the same thing around the clock. Eat a meal, digest it, excrete the remains, wait for the next meal. The composition of the community might shift with diet over weeks and months, but within a single day, we assumed, it was stable.

In 2014, Christoph Thaiss, then a graduate student in Eran Elinav’s laboratory at the Weizmann Institute, published a paper in *Cell* showing that this assumption was completely wrong [REF:thaiss2014]. Thaiss and colleagues sampled the gut microbiomes of mice at multiple time points across the 24-hour day and discovered that the composition oscillated. Specific bacterial taxa rose and fell in abundance over a daily rhythm. Specific microbial genes were transcribed more actively during some hours than others. The community was not static. It was cycling through daily phases that paralleled — and were driven by — the host’s feeding rhythm.

When the researchers disrupted the rhythm, by putting the mice on a jet-lag protocol (advancing the light cycle by 8 hours every three days), the microbial oscillations flattened and desynchron-

ised. The mice developed glucose intolerance and weight gain. When faecal microbiota from the jet-lagged mice were transplanted into germ-free mice on a normal schedule, the recipients also developed metabolic dysfunction — proof that the desynchronised microbiome was sufficient to cause the host metabolic phenotype. The team then studied two healthy human volunteers during actual transmeridian flights, and found evidence that the human gut microbiome was similarly desynchronised during jet lag and that the desynchrony correlated with the metabolic perturbations the volunteers experienced.

A follow-up paper from the same group in 2016 went further. Using mouse models, Thaïss and colleagues showed that the microbial oscillations were not just a passive consequence of feeding rhythms but an active driver of host physiology: the diurnal rhythms of microbial metabolite production shape the diurnal rhythms of gene expression in the liver and other tissues [REF:thaïss2016]. Your liver's daily schedule is not just set by your own clock. It is set, in part, by the metabolic output of the microbes in your gut, which in turn is set by when you eat.

This gave a microbial mechanism to a pattern that epidemiologists had been tracking for years. Shift workers have higher rates of metabolic syndrome, type 2 diabetes, cardiovascular disease, and some cancers than people who work regular hours. The usual explanation was that shift work disrupts sleep and stress systems. The Thaïss work added a new layer: shift work also disrupts the feeding rhythm, which disrupts the microbial rhythm, which disrupts the host metabolic rhythm. The damage is partly mediated by a microbial clock you cannot see.

Time-restricted eating

The obvious practical question is whether *when* you eat makes a measurable difference for people who are not shift workers. The growing body of human evidence suggests that it does, and the effect is large enough to take seriously.

Time-restricted eating is the practice of confining all daily food intake to a defined window — commonly eight, ten, or twelve hours — with the remaining portion of the 24-hour day spent not eating. It differs from the popular concept of “intermittent fasting” in that no calorie restriction is required; the person eats as much as they like within the window, but outside the window they drink only water, tea, or coffee without milk or sugar. In animal studies, time-restricted feeding produces improvements in glucose tolerance, lipid profiles, and body composition even when total calorie intake is held constant. In human studies, the evidence is more mixed but consistently points in the same direction.

A 2020 study by Zeb and colleagues randomised healthy adult men to a 16:8 time-restricted feeding pattern (8-hour eating window) or an ad libitum control for 25 days, and reported changes in gut microbiome composition, metabolic markers, and circadian rhythms in the restricted group that did not appear in the control [REF:zeb2020]. The effects were modest — this was a short trial in healthy participants, not a dramatic clinical intervention — but consistent with the broader picture.

More powerful evidence, at the population level, came from the French NutriNet-Santé cohort. In a 2023 paper, Palomar-Cros and colleagues analysed data from more than 100,000 adults whose eating times had been recorded prospectively [REF:palomar-cros2023]. They found that later first meal times and later last meal times were associated with higher cardiovascular disease risk, independent of total calorie intake and diet quality. Each additional hour of eating-window delay (eating later) was associated with meaningful increases in risk. Longer overnight fasts — essentially the space between dinner and breakfast — were associated with lower risk.

Put these threads together and you get the outline of a practical recommendation that, until recently, dietary advice did not contain. What matters is not only *what* you eat but also *when*. The simplest version of the recommendation is this: try to eat within a defined daily window, probably somewhere between 10 and 12 hours, and try not to eat in the hours immediately before bed. If you currently have your first coffee at 7 am and your last snack at 11 pm, you are eating across 16 hours, and your microbes — along with the circadian metabolism of your liver and your insulin-secreting cells — have no off-shift. Tightening the window to something like 8 am to 7 pm, or 9 am to 8 pm, gives the system a rest. For most people this is a free intervention: it does not cost anything, does not require changing what you eat, and can be adopted with relatively little disruption.

What the Mediterranean pattern had right all along

I want to return briefly to the Mediterranean pattern here, because one of its features that gets less attention than olive oil and tomatoes is the rhythm of its eating. In traditional Mediterranean cultures, meals were concentrated into three main events: a small breakfast, a significant mid-day meal eaten unhurriedly in company, and a light evening meal eaten relatively early. Snacking between meals was unusual. Eating late at night was unusual. The eating window was roughly 10 to 12 hours, not because anybody was practising time-restricted feeding but because that was the cultural pattern.

In other words, the pattern we now recommend for circadian reasons was already baked into the pattern we recommend for compositional reasons. This may be part of why long-term adherence to “the Mediterranean diet” as a lifestyle (not just a grocery list) produces results that tinkering with individual nutrients has historically not. The traditional pattern bundled the composition with the rhythm, and both parts may have been doing real work.

The person who wants the practical benefit without the full cultural immersion can take the simple advice: narrow the window, protect the overnight fast, avoid eating for two or three hours before sleep, and let the microbes have their daily rest.

20.11 Personalised Nutrition: Why “What Should I Eat?” Is the Wrong Question

The same banana, different people

In 2015, a team led by David Zeevi, Tal Korem, and Niv Zmora in the laboratories of Eran Segal and Eran Elinav at the Weizmann Institute published a study that I think should have changed the way popular nutrition is taught, and largely has not [REF:zeevi2015]. The study was called “Personalized Nutrition by Prediction of Glycemic Responses,” and it was, at the time, the largest and most detailed examination ever conducted of how real people respond to real food in their normal lives.

The design was this. Eight hundred healthy and pre-diabetic Israeli adults were recruited and equipped with continuous glucose monitors — the small skin-worn sensors that sample interstitial glucose every few minutes and produce a continuous record of blood glucose across days. For one week, the participants ate their normal diets, logged every meal in a smartphone app, and sent stool samples for microbiome sequencing. The researchers then standardised one meal per day — a specific bread, a specific breakfast cereal, a specific glucose drink — so that everybody was eating

the same thing at some point in the week. The team also collected anthropometric and clinical data: age, weight, height, blood tests, and so on. The total dataset included roughly 46,000 meals logged and sensor-tracked.

The analysis of that dataset produced a result so counter to the assumptions of public health nutrition that it is still underappreciated a decade later. When different people ate the *same* standardised meal — the same piece of bread, the same bowl of cereal, the same glucose drink — their blood glucose responses differed enormously. Person A's glucose might spike by 30 mg/dL after a slice of bread; person B's might spike by 10 mg/dL; person C's might spike by 90 mg/dL. These were not measurement errors. They were consistent, reproducible, within-person patterns that varied strikingly between people.

More interestingly, the variations were not predicted by any single simple variable. BMI, age, fasting glucose, and insulin resistance explained a little of the variance, but not most of it. The researchers then built a machine-learning model that incorporated all the measured variables — including the microbiome features — and asked whether the model could predict, for each individual, which foods would spike their glucose and which would not. It could. And the microbiome features were among the most important predictors. Particular gut bacterial taxa were associated with smoother glucose responses to particular foods. Other taxa were associated with sharper responses. The microbiome was carrying predictive information that the clinical variables were not.

What this means, in plain terms, is that two people can eat identical breakfasts and have entirely different metabolic experiences, and that the difference is partly a function of who their microbes are. “Bread causes a blood sugar spike” is true for some people and mostly false for others. “White rice is worse than brown rice” is a population-level statement that may not apply to any specific reader. The averaging has been hiding real variation, and the variation is not random — it is predictable, if you know enough about the individual.

PREDICT 1: the British twin study

The Weizmann work was done in a single population. It remained possible, in principle, that the striking individual variation was something about Israelis specifically, or about the particular measurement method, or about the chance characteristics of the sample. So in 2020, a large collaborative team — Tim Spector at King's College London, Andrew Chan at Harvard, Nicola Segata at Trento, and others — published the first results of a project called PREDICT 1, which was essentially the Weizmann design at larger scale and in a different population, with twins [REF:berry2020].

PREDICT 1 enrolled a thousand healthy adults, including hundreds of pairs of identical and non-identical twins, and subjected them to standardised meals under carefully controlled conditions, then tracked their free-living eating for two weeks. Continuous glucose, blood triglycerides, insulin, and other metabolic parameters were measured. The participants included twins because twin designs allow for partitioning of variance between genetic and non-genetic factors. If a trait varied more between non-identical twins than identical ones, you could quantify the genetic contribution.

The PREDICT 1 results replicated the Weizmann finding in a Western population and added precision to the partitioning. For postprandial glucose responses — the blood sugar curve after a meal — the contribution of genetics was small: perhaps 15 to 30 percent of the variance. Most of the individual variation was from non-genetic factors, including the microbiome, the person's current metabolic state, the time of day, the order in which components of the meal were eaten, the amount of sleep the person had had, and their habitual diet. For postprandial triglycerides, the pattern was

different — genetics contributed almost nothing to the variance, and the microbiome and lifestyle factors dominated almost completely.

A companion paper by Francesco Asnicar and colleagues, published in 2021 in *Nature Medicine*, specifically examined the microbiome arm of the PREDICT 1 data and reported that particular microbial taxa were associated with favourable and unfavourable cardiometabolic markers, and that habitual diet shaped microbiome composition in turn [REF:asnicar2021]. The team identified a “signature of 15 good microbes” that were associated with healthy plant-based diets and favourable blood markers, and a contrasting set of microbes associated with unhealthy processed diets and unfavourable markers. The key is that the relationships were mediated: diet shapes microbiome, microbiome shapes metabolism, metabolism shapes disease risk.

Where the evidence stands

Taken together, the Weizmann and PREDICT studies tell us that “one size fits all” nutrition is not a useful framework for individual-level advice. The same food produces different effects in different people, the differences are partly predictable from the microbiome, and the predictive models are good enough to be commercially interesting — several companies (including the ones descended from the Weizmann and PREDICT teams) now offer microbiome-based personalised nutrition services on a subscription basis.

But we should be careful here. A 2019 review by Kolodziejczyk, Zheng, and Elinav, published in *Nature Reviews Microbiology*, assessed the state of evidence for personalised nutrition and reached a balanced conclusion: the science is promising but the clinical use is still early [REF:kolodziejczyk2019]. The models predict group-level average responses better than individual-level variation. The tests are expensive. The actionable output — “eat more of X and less of Y” — is useful for some people in some contexts but does not, on current evidence, outperform general principles (more fibre, more plant diversity, less ultra-processed food) for most readers most of the time.

My own view, as a physician who watches how new technologies travel from research to clinic, is this. The scientific finding — that individual variation in response to food is real, substantial, and partly microbiome-mediated — is robust and important. The commercial translation — “send us a stool sample and we’ll tell you what to eat for £300” — is premature. If you have a specific clinical problem that is not responding to general advice (treatment-resistant type 2 diabetes, recurrent metabolic syndrome, stubborn weight gain despite reasonable eating), a personalised approach guided by continuous glucose monitoring and microbiome testing may add value. If you are a healthy reader trying to eat well, the general principles of this chapter — fibre, plant diversity, fermented foods, Mediterranean pattern, ultra-processed minimisation, time-restricted window — will do more, more cheaply, than any commercial test will.

The more interesting implication of the personalised nutrition literature, I think, is not that you should rush to buy a test. It is that you should stop trusting any single-food headline. When the Sunday newspaper tells you that “bananas cause blood sugar spikes” or “bananas are a perfect low-glycemic snack,” both statements are true and both statements are false, depending on which reader they are addressed to. The same goes for eggs, potatoes, white rice, oats, dairy, legumes, and almost every food that gets held up or held down in popular discussion. *You* are part of the equation, and the part of you that matters most for this particular question is sitting in your colon.

20.12 The Controversies, Met Head-On

I have avoided taking sides in the big contemporary food controversies until now, because the evidence-based case for the practical advice of this chapter does not depend on any of them. Fibre, plant diversity, fermented foods, Mediterranean pattern, minimising ultra-processed food, narrowing the eating window — all of that holds up regardless of where you stand on gluten, FODMAPs, lectins, or meat. But readers who have made it this far are entitled to a direct engagement with the positions they encounter on social media and in popular books, and this section is where I give them.

The structure of each of the following sub-sections is the same. I will state the claim as its adherents make it, describe what the best available evidence actually says, distinguish the kernel of truth from the exaggeration, and end with a practical recommendation. I will do this without sarcasm, because most of the people who hold these positions have arrived at them honestly — often because they felt unwell, tried something, felt better, and concluded that the thing they tried was the answer. That is a reasonable way to discover something. It is a less reasonable way to prove it.

Gluten

The claim made by the popular gluten-free movement is that wheat and other gluten-containing grains are, for a significant fraction of the population, a cause of bloating, fatigue, brain fog, joint pain, and a variety of systemic symptoms, and that the remedy is avoidance of all gluten-containing foods.

The evidence-based picture is more precise and more interesting. Celiac disease is a real, specific, autoimmune condition in which the body's own immune system attacks the lining of the small intestine in response to gluten exposure. It affects roughly 1 percent of most populations, it can be diagnosed reliably with blood tests and a small-bowel biopsy, and it requires strict lifelong gluten avoidance. If you have been formally diagnosed with celiac disease, the advice in this chapter about whole grains and fermented bread does not apply to you in the same form, and gluten-free alternatives are medically necessary rather than optional.

Non-celiac wheat sensitivity is also real, although how common it is depends on how it is defined. A proportion of people who do not have celiac disease but who report symptomatic improvement when they avoid wheat appear to have genuine sensitivity to *something* in wheat. The question that the research of the last decade has been trying to answer is: sensitivity to what, exactly?

The most carefully designed trial to address this question came from Jessica Biesiekierski, Peter Gibson, and colleagues at Monash University in 2013, and it tells a story that is not what most readers expect [REF:biesiekierski2013]. The Monash group recruited participants who self-identified as having non-celiac gluten sensitivity and whose symptoms had improved on a gluten-free diet. In a controlled crossover trial, participants were put on a low-FODMAP diet (that is, a diet low in fermentable oligosaccharides, disaccharides, monosaccharides, and polyols — we will come back to FODMAPs in a moment) and then randomised to receive either pure gluten, pure whey protein, or placebo, in a blinded fashion, added to an otherwise low-FODMAP background diet.

The result was striking. Participants' symptoms improved on the low-FODMAP diet. When they were then challenged with pure gluten, they reported no more symptoms than when challenged with whey protein or placebo. Gluten, when separated from the FODMAP content of wheat, did not reproduce the symptoms. FODMAP reduction, which was part of eliminating wheat, did. The trial suggested that a substantial fraction of the people who self-identify as "gluten sensitive" are

actually sensitive to the fermentable carbohydrates in wheat — particularly fructans — rather than to gluten itself.

The practical implication is important. If you feel better when you avoid wheat, you are not imagining it — something is probably going on. But the thing that is going on is more likely to be a FODMAP issue than a gluten issue, and the appropriate response is not necessarily to go gluten-free forever; it is to identify which fermentable carbohydrates you react to and consider long-fermented sourdough bread, which, because the yeasts and bacteria pre-digest much of the fructan content during the long rise, is often tolerated by people who cannot eat commercial bread.

A second piece of evidence is worth noting for anyone who has been tempted to adopt gluten avoidance as a general health measure rather than in response to specific symptoms. Benjamin Lebwohl and colleagues, in a 2017 *BMJ* paper, analysed long-term dietary and health data from over 100,000 American health professionals across more than two decades [REF:lebwohl2017]. They found that among people without celiac disease, long-term avoidance of gluten was associated with *higher*, not lower, cardiovascular disease risk, most plausibly because it was associated with reduced whole-grain intake. The gluten-free aisle of the supermarket is dominated by refined starches, added sugars, and industrial emulsifiers, and systematically replacing whole wheat with commercial gluten-free substitutes is likely to produce a nutritionally worse diet, not a better one.

So: if you have celiac disease, avoid gluten strictly. If you suspect non-celiac gluten sensitivity, do not assume gluten is the culprit; consider the FODMAP explanation, try long-fermented sourdough, and get formally assessed by a gastroenterologist or dietitian before committing to indefinite avoidance. If you have no symptoms and are considering “going gluten-free for health,” the evidence does not support it, and it may worsen your cardiovascular risk.

FODMAPs and the low-FODMAP diet

FODMAP stands for fermentable oligosaccharides, disaccharides, monosaccharides, and polyols — a collection of short-chain carbohydrates that are poorly absorbed in the small intestine, reach the colon, and are rapidly fermented by gut bacteria, producing gas and drawing water into the lumen. In people with irritable bowel syndrome (IBS), this gas and osmotic water shift can trigger bloating, pain, and altered bowel habit. The low-FODMAP diet, developed at Monash University, restricts dietary FODMAPs to a minimum and has become one of the best-supported dietary interventions for IBS symptom control.

The evidence for its efficacy in IBS is strong. Randomised trials, summarised in a 2017 review by Staudacher and Whelan, show symptom improvement in approximately 50 to 75 percent of IBS patients within four weeks of starting the strict elimination phase [REF:staudacher2017]. This is better than most pharmacological treatments for IBS, and the effect is generally rapid and clinically meaningful.

But the low-FODMAP diet has a problem from the microbiome point of view, and it is the same problem the fibre section of this chapter has been warning about. FODMAPs are fermentable substrates for the gut microbes — they are, in effect, a subset of microbiota-accessible carbohydrates. Stopping them reduces the substrate supply to the bacteria that ferment them, particularly *Bifidobacterium*, one of the most consistently beneficial genera in the human gut. A study by Halmos and colleagues in 2014 showed that a low-FODMAP diet for just three weeks significantly reduced faecal *Bifidobacterium* and total SCFA production [REF:halmos2014]. The short-term cost is real.

This is why the low-FODMAP diet is not meant to be a long-term way of eating, and when it is pre-

scribed by a knowledgeable dietitian it is explained as a three-phase process: (1) strict elimination for two to six weeks to achieve symptom control, (2) systematic reintroduction of one FODMAP class at a time to identify which ones trigger symptoms, and (3) long-term personalised restriction of the specific problematic FODMAPs only, with all tolerated FODMAPs returned to the diet. The third phase is the destination. The first phase is a diagnostic tool. People who get stuck in the first phase — who adopt the low-FODMAP elimination as a permanent lifestyle — are trading symptom control for accumulating microbiome impoverishment, and the trade may not be worth it long-term.

A related and interesting finding comes from Bennet and colleagues in 2018, who showed that baseline microbiome composition in IBS patients predicts who will respond to the low-FODMAP diet [REF:bennet2018]. Some IBS patients have microbial profiles that suggest they will respond well; others will not. This is another example of the personalisation theme — the same intervention, applied to different people, produces different results, and the difference is partly predictable from the microbiome.

Practical recommendation: if you have IBS and have not yet tried a properly supervised low-FODMAP diet, it is worth doing — under the guidance of a dietitian who understands the three-phase structure. If you have tried it and got stuck in the elimination phase, work with a dietitian to reintroduce systematically. If you do not have IBS, the low-FODMAP diet is not for you, and long-term FODMAP restriction in healthy people is likely to harm rather than help the gut microbiome.

Lectins

The claim, popularised by a series of bestselling books, is that lectins — a class of proteins found in many plants, particularly legumes, grains, nightshade vegetables, and some fruits — are a hidden cause of widespread chronic illness, and that a “lectin-free” diet is the path to weight loss, reduced inflammation, and improved health.

The evidence base for this claim is, to put it charitably, thin. Lectins are real proteins, and some of them — notably the ricin of castor beans — are genuinely toxic. Raw kidney beans contain a lectin called phytohaemagglutinin that causes acute gastrointestinal distress if eaten in quantity, which is why nobody eats raw kidney beans; a brief period of boiling completely denatures the lectin, and properly cooked beans are harmless. Raw wheat germ contains wheat germ agglutinin, which has been shown in laboratory experiments to interact with intestinal cells, but the quantity in normal bread and the effect of cooking reduce this to biological irrelevance.

The broader “lectin theory” extrapolates from these edge cases to the claim that lectins in ordinary, properly prepared, commonly consumed foods are causing harm. The epidemiological evidence runs powerfully against this claim. Legumes are one of the best-validated predictors of long-term health in every long-lived population that has been studied — the Blue Zone populations of Okinawa, Sardinia, and Nicoya all eat legumes in significant quantity and live exceptionally long lives. Whole grains are associated with lower all-cause mortality in every large cohort study that has measured them. Nightshade vegetables (tomatoes, peppers, eggplants, potatoes) are central to several of the healthiest traditional diets in the world. If lectins were causing the damage the theory posits, these associations would go the other way, and they do not.

From the microbiome point of view, the lectin theory is even less plausible, because the foods it demonises are, almost without exception, high in fibre and polyphenols and are precisely the foods we

have spent this chapter arguing are essential for microbiome health. Removing them would starve the very bacterial community that the rest of the popular “gut health” literature — including the same authors who promote lectin avoidance — claims to want to nurture. The position is internally inconsistent.

Practical recommendation: do not adopt lectin avoidance. Cook your beans properly, eat your whole grains, and let your tomatoes be. If you have a specific, formally diagnosed food intolerance, address that; do not generalise from it to an entire food category.

Carnivore

The most extreme of the current dietary movements is the carnivore diet: an eating pattern that eliminates all plant foods and consists exclusively of animal products, most commonly beef, eggs, butter, and small amounts of salt. Adherents report, often in dramatic terms, resolution of chronic symptoms — autoimmune disease, depression, inflammation, joint pain — on the elimination of plants.

I want to take these reports seriously, because dismissing them outright would be wrong. Some of the people who feel better on a carnivore diet have real symptoms that were not helped by conventional medicine, and their experience of improvement is real to them. The question is what the improvement actually represents.

The most plausible explanation — and this is a hypothesis, not an established fact — is that for a subset of these individuals, what appears to be an improvement on “removing plants” is actually an improvement on removing a specific subset of plant compounds to which they were reacting. If that subset included the fermentable carbohydrates that cause IBS, the oxalates that precipitate in some people’s joints or kidneys, the histamine-liberating foods that provoke mast-cell reactions, the nightshades some people with autoimmune conditions are sensitive to, and perhaps the phytate-chelated minerals that are poorly absorbed in some gut profiles — then removing all of them at once by removing all plants could produce the improvement without the improvement being specifically due to “animal foods.” The same improvement, in those individuals, might well be achievable by a carefully designed plant-inclusive diet that happens to avoid their specific triggers.

The problem with the carnivore diet as a long-term strategy is that we have almost no long-term data. The evidence base consists of testimonials, short-term observational reports, and theoretical argument. What we know about gut microbiome biology suggests that a strict and sustained plant-free diet will produce a microbiome with markedly reduced diversity, near-absent fibre-fermenting capacity, shrunk SCFA production, and correspondingly reduced butyrate fuel for colonocytes. We have already discussed what the David 2014 diet-switch experiment showed in humans: dramatic shifts within days towards animal-diet-typical microbial profiles, including expansion of bile-tolerant and sulphide-producing organisms [REF:david2014]. We have already discussed what the TMAO literature shows about heavy meat intake and cardiovascular risk [REF:koeth2013]. We have already discussed the Sonnenburg extinction experiments and what low-MAC diets do to diversity across generations [REF:sonnenburg2016]. None of these are reassuring about a prolonged carnivore pattern.

My practical recommendation is this. If you feel unwell on your current diet and have not been able to get good answers from conventional medicine, the carnivore experiment is not unreasonable as a short-term diagnostic tool — two or three weeks of strict elimination, followed by systematic reintroduction of plant categories one at a time, might help you identify what you actually react

to. This is, in essence, the same diagnostic logic as the low-FODMAP diet, just starting from a different end. What I would not recommend is the indefinite maintenance of the strict elimination phase as a long-term way of eating, on testimonial evidence alone, against a body of mechanistic and epidemiological evidence pointing the other way. The upside — that you personally will be an exception to everything we know about long-term nutrition and microbiome biology — is possible but uncertain. The downside — that you will de-train a microbial community across years in ways that cannot be easily undone — is documented.

A note on certainty

I have stated positions in this section more directly than I usually would in a book like this, because I think the reader is entitled to them. I want to be equally direct that in each of these cases, the evidence base has gaps and the scientific conversation continues. If new high-quality trials change the picture — if a properly designed long-term carnivore trial showed benefits we did not predict, or if the FODMAP explanation for non-celiac sensitivity turned out to be only part of the story — I would update my position, and a later edition of this book would say something different. What I have tried to give you is the current best reading of the best available evidence, not a pronouncement from on high. Take it as such, and watch the literature.

20.13 What This Means for Tuesday Dinner

Here is the practical summary of the chapter, tiered by the strength of the evidence, so that a reader who has read only this section has the useful distillation.

The strongest evidence: things to do

Eat more fibre. Work up to 30 grams or more per day. Choose a variety of sources — whole grains, legumes, vegetables, fruits, nuts, seeds — rather than relying on a single fibre type. If you are currently at 12 to 15 grams per day (which is most Western adults), doubling your intake over a few weeks is one of the single most evidence-based changes you can make.

Aim for thirty different plant species per week. Count everything: vegetables, fruits, whole grains, legumes, nuts, seeds, herbs, spices, coffee, tea, dark chocolate. Write them down for a week if you find it hard to believe you are reaching or not reaching the target. The number itself is less important than the habit of variety.

Eat real fermented foods most days. A small serving — a spoonful of yogurt with live cultures, a forkful of kimchi or sauerkraut from the refrigerated section, a cup of kefir, a piece of aged cheese, a small glass of kombucha — at most meals. Read labels; avoid the pasteurised impostors. The Stanford evidence suggests this may be the single intervention most likely to reduce inflammatory tone in the healthy adult.

Adopt a Mediterranean-style pattern. Plants at the centre of the plate, olive oil as the dominant fat, small portions of fish and meat a few times a week, legumes and whole grains at most meals, herbs and spices in abundance, fruit for dessert, water and unsweetened drinks as the default beverage. This is not a diet in the sense of a temporary restriction; it is a cuisine, and there are a hundred versions of it from a hundred cultures.

Eat within a defined daily window. Somewhere between 10 and 12 hours is a reasonable target for most people. Protect the overnight fast; avoid eating in the two or three hours before sleep.

Strong evidence: things to reduce

Reduce ultra-processed foods. The category includes almost everything in brightly coloured packaging with long ingredient lists and names you cannot pronounce. Perfect elimination is not required and often not practical; steady reduction is. Aim to get the majority of your calories from foods recognisable as foods. A useful heuristic: if it was not food a hundred years ago, be suspicious.

Reduce dietary emulsifiers. Read labels for carboxymethylcellulose (E466), polysorbate 80 (E433), carrageenan, and mono- and diglycerides. These are concentrated in processed ice creams, commercial breads, processed cheeses, plant milks, salad dressings, and ready meals. You cannot avoid them entirely and probably do not need to, but reducing daily exposure is sensible.

Reduce non-nutritive sweeteners. Particularly saccharin and sucralose, the two with the clearest evidence of microbiome-mediated effects in the Suez 2022 human trial. Aspartame and stevia have less conclusive evidence but also produced microbiome changes in the same study and should be viewed with modest caution. Water, unsweetened tea, unsweetened coffee, and small amounts of real sugar are all better choices than constant diet-sweetened drinks.

Reduce red and processed meat to a few times a week at most. Not because meat is poison but because heavy long-term intake trains a microbial community that produces more TMAO and secondary bile acid metabolites than most of us want. A small serving of good meat, a few times a week, as part of a plant-heavy meal, is consistent with everything this chapter has argued for.

Promising but not yet prescription-grade

Personalised microbiome-based meal planning. The science is interesting and improving. The commercial services are expensive and the output is not yet clearly better than general principles for most people. Wait, for now, unless you have a specific clinical problem that general advice has not solved.

Polyphenol supplementation. Eat the whole foods; supplementation with isolated polyphenols has produced inconsistent results in trials, and the metabotype story makes it clear that the systemic effect depends on who is eating.

Extreme time-restricted feeding windows (shorter than 8 hours). The evidence for a 10- to 12-hour window is reasonable. The evidence for shorter windows is less clear, and very short windows (4 to 6 hours) may cause problems for some people, particularly women, shift workers, and people with blood sugar regulation issues. Start gentle.

Not supported by evidence

Gluten-free eating for people without celiac disease or diagnosed wheat allergy. Does not help; may harm.

Lectin avoidance. Eliminates some of the most strongly evidence-based foods on the planet on the basis of a theory not supported by human epidemiology.

Strict long-term carnivore eating. Short-term diagnostic use may have a role. Long-term maintenance is not supported by existing evidence and conflicts with much of what we know about microbiome biology.

Fad diets generally. If a diet is named after its inventor, promises dramatic results in weeks, demands a book purchase to understand fully, and excludes one or more major food groups, be cautious. The evidence base for any of these, compared with the traditional Mediterranean pattern or any whole-foods plant-heavy approach, is comparatively thin.

The closing image

Dinner is a daily act of ecology. When you choose what to put on your plate, you are not only feeding yourself. You are feeding a forest — a living, breathing, metabolising community of trillions of organisms whose daily output of small molecules shapes how your own cells function, how your immune system responds, how your gut wall holds together, and in the long run how well you age. Most of them you will never see and never think about. But they will process every meal you eat, and what they make of it depends, every day, on what you choose to give them.

The garden metaphor I began this chapter with has one last application. A gardener who tends a piece of ground for decades does not produce miraculous results through any single intervention. She produces them through the accumulated consequence of small, consistent choices across many seasons. The right compost. The right watering. The right crop rotation. The right attention to the slow signals of the soil. None of it is dramatic. All of it matters. And at the end of twenty years, one gardener has a rich, productive, resilient plot and another has depleted earth, and the difference is the sum of daily decisions that neither of them noticed mattering at the time.

Your gut is the ground you have to work with. Feed it well, and its inhabitants will pay you back across a lifetime.

20.14 Looking Ahead

Diet is the single most powerful daily lever most of us have over our microbiome. But it is not the only lever, and it sits inside a much larger web of environmental and lifestyle factors that shape the community of organisms we carry. The air you breathe, the water you drink, the dirt your children play in, the pets in your home, the people you live with, the medications you take, the sleep you get, the stress you carry, the exercise you do or do not do — all of them leave fingerprints on the ecosystem inside you. Chapter 21 widens the lens to look at those other inputs, and asks how the rest of the way we live — beyond what we put on the plate — is shaping the microbial communities that are, by now, unmistakably part of who we are.

Key References

Asnicar F, Berry SE, Valdes AM, et al. (2021). Microbiome connections with host metabolism and habitual diet from 1,098 deeply phenotyped individuals. *Nature Medicine* 27(2):321-332. doi:10.1038/s41591-020-01183-8. [REF:asnicar2021]

- Bennet SMP, Böhn L, Störsrud S, et al. (2018). Multivariate modelling of faecal bacterial profiles and food intake in irritable bowel syndrome. *Gut* 67(5):872-881. doi:10.1136/gutjnl-2016-313128. [REF:bennet2018]
- Berry SE, Valdes AM, Drew DA, et al. (2020). Human postprandial responses to food and potential for precision nutrition. *Nature Medicine* 26(6):964-973. doi:10.1038/s41591-020-0934-0. [REF:berry2020]
- Bielik V, Kolisek M (2021). Bioaccessibility and bioavailability of minerals in relation to a healthy gut microbiome. *International Journal of Molecular Sciences* 22(13):6803. doi:10.3390/ijms22136803. [REF:bielik2021]
- Biesiekierski JR, Peters SL, Newnham ED, Rosella O, Muir JG, Gibson PR (2013). No effects of gluten in patients with self-reported non-celiac gluten sensitivity after dietary reduction of fermentable, poorly absorbed, short-chain carbohydrates. *Gastroenterology* 145(2):320-328.e3. doi:10.1053/j.gastro.2013.04.051. [REF:biesiekierski2013]
- Chassaing B, Koren O, Goodrich JK, et al. (2015). Dietary emulsifiers impact the mouse gut microbiota promoting colitis and metabolic syndrome. *Nature* 519(7541):92-96. doi:10.1038/nature14232. [REF:chassaing2015]
- Chassaing B, Compher C, Bonhomme B, et al. (2022). Randomized controlled-feeding study of dietary emulsifier carboxymethylcellulose reveals detrimental impacts on the gut microbiota and metabolome. *Gastroenterology* 162(3):743-756. doi:10.1053/j.gastro.2021.11.006. [REF:chassaing2022]
- Cortés-Martín A, Selma MV, Tomás-Barberán FA, González-Sarrias A, Espín JC (2020). Where to look into the puzzle of polyphenols and health? The postbiotics and gut microbiota associated with human metabolotypes. *Molecular Nutrition & Food Research* 64(9):e1900952. doi:10.1002/mnfr.201900952. [REF:cortes-martin2020]
- David LA, Maurice CF, Carmody RN, et al. (2014). Diet rapidly and reproducibly alters the human gut microbiome. *Nature* 505(7484):559-563. doi:10.1038/nature12820. [REF:david2014]
- Davis DR, Epp MD, Riordan HD (2004). Changes in USDA food composition data for 43 garden crops, 1950 to 1999. *Journal of the American College of Nutrition* 23(6):669-682. doi:10.1080/07315724.2004.10719409. [REF:davis2004]
- Deehan EC, Mocanu V, Madsen KL (2024). Effects of dietary fibre on metabolic health and obesity. *Nature Reviews Gastroenterology & Hepatology* 21(5):301-318. doi:10.1038/s41575-023-00891-z. [REF:deehan2024]
- Desai MS, Seekatz AM, Koropatkin NM, et al. (2016). A dietary fiber-deprived gut microbiota degrades the colonic mucus barrier and enhances pathogen susceptibility. *Cell* 167(5):1339-1353.e21. doi:10.1016/j.cell.2016.10.043. [REF:desai2016]
- Devkota S, Wang Y, Musch MW, et al. (2012). Dietary-fat-induced taurocholic acid promotes pathobiont expansion and colitis in *Il10*^{-/-} mice. *Nature* 487(7405):104-108. doi:10.1038/nature11225. [REF:devkota2012]
- Furusawa Y, Obata Y, Fukuda S, et al. (2013). Commensal microbe-derived butyrate induces the differentiation of colonic regulatory T cells. *Nature* 504(7480):446-450. doi:10.1038/nature12721. [REF:furusawa2013]

- Ghosh TS, Rampelli S, Jeffery IB, et al. (2020). Mediterranean diet intervention alters the gut microbiome in older people reducing the risk of frailty: results from the NU-AGE 1-year dietary intervention across five European countries. *Gut* 69(7):1218-1228. doi:10.1136/gutjnl-2019-319654. [REF:ghosh2020]
- Halmos EP, Christophersen CT, Bird AR, Shepherd SJ, Gibson PR, Muir JG (2014). Diets that differ in their FODMAP content alter the colonic luminal microenvironment. *Gut* 63(1):93-100. doi:10.1136/gutjnl-2014-307264. [REF:halmos2014]
- Koeth RA, Wang Z, Levison BS, et al. (2013). Intestinal microbiota metabolism of L-carnitine, a nutrient in red meat, promotes atherosclerosis. *Nature Medicine* 19(5):576-585. doi:10.1038/nm.3145. [REF:koeth2013]
- Koh A, De Vadder F, Kovatcheva-Datchary P, Bäckhed F (2016). From dietary fiber to host physiology: short-chain fatty acids as key bacterial metabolites. *Cell* 165(6):1332-1345. doi:10.1016/j.cell.2016.05.041. [REF:koh2016]
- Kolodziejczyk AA, Zheng D, Elinav E (2019). Diet-microbiota interactions and personalized nutrition. *Nature Reviews Microbiology* 17(12):742-753. doi:10.1038/s41579-019-0256-8. [REF:kolodziejczyk2019]
- Lebwohl B, Cao Y, Zong G, et al. (2017). Long term gluten consumption in adults without celiac disease and risk of coronary heart disease: prospective cohort study. *BMJ* 357:j1892. doi:10.1136/bmj.j1892. [REF:lebwohl2017]
- Lönnerdal B (2017). Excess iron intake as a factor in growth, infections, and development of infants and young children. *American Journal of Clinical Nutrition* 106(Suppl 6):1681S-1687S. doi:10.3945/ajcn.117.156042. [REF:lonnerdal2017]
- Mao Q, Manservigi F, Panzacchi S, et al. (2018). The Ramazzini Institute 13-week pilot study on glyphosate and Roundup administered at human-equivalent dose to Sprague Dawley rats: effects on the microbiome. *Environmental Health* 17(1):50. doi:10.1186/s12940-018-0394-x. [REF:mao2018]
- Marco ML, Heeney D, Binda S, et al. (2017). Health benefits of fermented foods: microbiota and beyond. *Current Opinion in Biotechnology* 44:94-102. doi:10.1016/j.copbio.2016.11.010. [REF:marco2017]
- McDonald D, Hyde E, Debelius JW, et al.; American Gut Consortium (2018). American Gut: an open platform for citizen science microbiome research. *mSystems* 3(3):e00031-18. doi:10.1128/mSystems.00031-18. [REF:mcdonald2018]
- Meslier V, Laiola M, Roager HM, et al. (2020). Mediterranean diet intervention in overweight and obese subjects lowers plasma cholesterol and causes changes in the gut microbiome and metabolome independently of energy intake. *Gut* 69(7):1258-1268. doi:10.1136/gutjnl-2019-320438. [REF:meslier2020]
- Mesnage R, Teixeira M, Mandrioli D, et al. (2021). Use of shotgun metagenomics and metabolomics to evaluate the impact of glyphosate or Roundup MON 52276 on the gut microbiota and serum metabolome of Sprague-Dawley rats. *Environmental Health Perspectives* 129(1):17005. doi:10.1289/EHP6990. [REF:mesnage2021]
- Mesnage R, Bowyer RCE, El Balkhi S, et al. (2022). Impacts of dietary exposure to pesticides on faecal microbiome metabolism in adult twins. *Environmental Health* 21(1):46.

doi:10.1186/s12940-022-00860-0. [REF:mesnage2022twins]

Motta EVS, Raymann K, Moran NA (2018). Glyphosate perturbs the gut microbiota of honey bees. *Proceedings of the National Academy of Sciences USA* 115(41):10305-10310. doi:10.1073/pnas.1803880115. [REF:motta2018]

Paganini D, Uyoga MA, Kortman GAM, et al. (2017). Prebiotic galacto-oligosaccharides mitigate the adverse effects of iron fortification on the gut microbiome: a randomised controlled study in Kenyan infants. *Gut* 66(11):1956-1967. doi:10.1136/gutjnl-2017-314418. [REF:paganini2017]

Paganini D, Uyoga MA, Kortman GAM, et al. (2018). Iron-containing micronutrient powders modify the effect of oral antibiotics on the infant gut microbiome and increase post-antibiotic diarrhoea risk: a controlled study in Kenya. *Gut* 68(4):645-653. doi:10.1136/gutjnl-2018-317399. [REF:paganini2018]

Palomar-Cros A, Andreeva VA, Fezeu LK, et al. (2023). Dietary circadian rhythms and cardiovascular disease risk in the prospective NutriNet-Sant  cohort. *Nature Communications* 14(1):7899. doi:10.1038/s41467-023-43444-3. [REF:palomar-cros2023]

Ranaivo H, Thirion F, B ra-Maillet C, et al. (2022). Increasing the diversity of dietary fibers in a daily-consumed bread modifies gut microbiota and metabolic profile in subjects at cardiometabolic risk. *Gut Microbes* 14(1):2044722. doi:10.1080/19490976.2022.2044722. [REF:ranaivo2022]

Reynolds A, Mann J, Cummings J, Winter N, Mete E, Te Morenga L (2019). Carbohydrate quality and human health: a series of systematic reviews and meta-analyses. *Lancet* 393(10170):434-445. doi:10.1016/S0140-6736(18)31809-9. [REF:reynolds2019]

Schnorr SL, Candela M, Rampelli S, et al. (2014). Gut microbiome of the Hadza hunter-gatherers. *Nature Communications* 5:3654. doi:10.1038/ncomms4654. [REF:schnorr2014]

Smith PM, Howitt MR, Panikov N, et al. (2013). The microbial metabolites, short-chain fatty acids, regulate colonic Treg cell homeostasis. *Science* 341(6145):569-573. doi:10.1126/science.1241165. [REF:smith2013]

Smits SA, Leach J, Sonnenburg ED, et al. (2017). Seasonal cycling in the gut microbiome of the Hadza hunter-gatherers of Tanzania. *Science* 357(6353):802-806. doi:10.1126/science.aan4834. [REF:smits2017]

Sonnenburg ED, Sonnenburg JL (2014). Starving our microbial self: the deleterious consequences of a diet deficient in microbiota-accessible carbohydrates. *Cell Metabolism* 20(5):779-786. doi:10.1016/j.cmet.2014.07.003. [REF:sonnenburg2014]

Sonnenburg ED, Smits SA, Tikhonov M, Higginbottom SK, Wingreen NS, Sonnenburg JL (2016). Diet-induced extinctions in the gut microbiota compound over generations. *Nature* 529(7585):212-215. doi:10.1038/nature16504. [REF:sonnenburg2016]

Staudacher HM, Whelan K (2017). The low FODMAP diet: recent advances in understanding its mechanisms and efficacy in IBS. *Gut* 66(8):1517-1527. doi:10.1136/gutjnl-2017-313750. [REF:staudacher2017]

Suez J, Korem T, Zeevi D, et al. (2014). Artificial sweeteners induce glucose intolerance by altering the gut microbiota. *Nature* 514(7521):181-186. doi:10.1038/nature13793. [REF:suez2014]

Suez J, Cohen Y, Vald s-Mas R, et al. (2022). Personalized microbiome-driven effects of non-nutritive sweeteners on human glucose tolerance. *Cell* 185(18):3307-3328.e19. doi:10.1016/j.cell.2022.07.016.

[REF:suez2022]

Taylor BC, Lejzerowicz F, Poirel M, et al. (2020). Consumption of fermented foods is associated with systematic differences in the gut microbiome and metabolome. *mSystems* 5(2):e00901-19. doi:10.1128/mSystems.00901-19. [REF:taylor2020]

Thaiss CA, Zeevi D, Levy M, et al. (2014). Transkingdom control of microbiota diurnal oscillations promotes metabolic homeostasis. *Cell* 159(3):514-529. doi:10.1016/j.cell.2014.09.048. [REF:thaiss2014]

Thaiss CA, Levy M, Korem T, et al. (2016). Microbiota diurnal rhythmicity programs host transcriptome oscillations. *Cell* 167(6):1495-1510.e12. doi:10.1016/j.cell.2016.11.003. [REF:thaiss2016]

Tomás-Barberán FA, González-Sarriás A, García-Villalba R, et al. (2017). Urolithins, the rescue of “old” metabolites to understand a “new” concept: Metabotypes as a nexus among phenolic metabolism, microbiota dysbiosis, and host health status. *Molecular Nutrition & Food Research* 61(1). doi:10.1002/mnfr.201500901. [REF:tomas-barberan2017]

Turroni S, Fiori J, Rampelli S, et al. (2016). Fecal metabolome of the Hadza hunter-gatherers: a host-microbiome integrative view. *Scientific Reports* 6:32826. doi:10.1038/srep32826. [REF:turroni2016]

Walsh L, Hill C, Ross RP (2023). Impact of glyphosate (Roundup) on the composition and functionality of the gut microbiome. *Gut Microbes* 15(2):2263935. doi:10.1080/19490976.2023.2263935. [REF:walsh2023]

Wang Z, Klipfell E, Bennett BJ, et al. (2011). Gut flora metabolism of phosphatidylcholine promotes cardiovascular disease. *Nature* 472(7341):57-63. doi:10.1038/nature09922. [REF:wang2011]

Wastyk HC, Fragiadakis GK, Perelman D, et al. (2021). Gut-microbiota-targeted diets modulate human immune status. *Cell* 184(16):4137-4153.e14. doi:10.1016/j.cell.2021.06.019. [REF:wastyk2021]

Wu GD, Chen J, Hoffmann C, et al. (2011). Linking long-term dietary patterns with gut microbial enterotypes. *Science* 334(6052):105-108. doi:10.1126/science.1208344. [REF:wu2011]

Zeb F, Wu X, Chen L, et al. (2020). Effect of time-restricted feeding on metabolic risk and circadian rhythm associated with gut microbiome in healthy males. *British Journal of Nutrition* 123(11):1216-1226. doi:10.1017/S0007114519003428. [REF:zeb2020]

Zeevi D, Korem T, Zmora N, et al. (2015). Personalized nutrition by prediction of glycemic responses. *Cell* 163(5):1079-1094. doi:10.1016/j.cell.2015.11.001. [REF:zeevi2015]

Zinöcker MK, Lindseth IA (2018). The Western diet-microbiome-host interaction and its role in metabolic disease. *Nutrients* 10(3):365. doi:10.3390/nu10030365. [REF:zinocker2018]

End of Chapter 20 draft.

Chapter 21: The Other Levers — How the Rest of How We Live Shapes the Ecosystem Inside Us

21.1 The Garden Has Weather

At the end of the previous chapter we left the reader with a practical promise: that the single most powerful daily lever most of us have over our microbiome is what goes onto the plate. Everything in Chapter 20 — the thirty-plant rule, the short-chain fatty acids, the mucus layer, the rediscovered importance of variety over quantity — was built on that lever. If the only message a reader remembers from this book is *eat more kinds of plants*, the book will have done its job.

But a gardener who tends the soil but ignores the weather will have a poor garden. And our microbiome, like any ecosystem, has weather. Some of it we control; some of it we do not. Some of it we invented in the last hundred years without realising we had invented a climate. This chapter is about those other inputs — the temperature of the room, the light on your eyelids at midnight, the dust that used to be on the furniture, the dog that isn't in the house, the stranger on the tram, the alarm clock that goes off when the rest of your organs would rather be asleep. Most of them are not things you think about at all. That is precisely why they are worth a chapter.

I should warn you at the outset that the evidence here is uneven. For some of the levers — artificial light and the circadian rhythm, for example, or the exposure of infants to household pets — the data are now strong enough to support firm statements. For others — what a HEPA filter in your bedroom might be doing to the airborne microbes you would otherwise inhale, or whether decades of living in a thermally constant twenty-two degrees has narrowed the range of microbial conditions your body experiences — we are still at the stage of reasonable mechanistic speculation rather than settled science. I will flag the difference as we go. Where I am guessing, I will say so. Where the literature is thin, I will say that too.

One more thing, before we start. Some of the topics in this chapter arrived in my notebook only very late in the writing of this book — not because they are unimportant, but because they fell outside the usual frame of microbiome research, which has been dominated by the diet–gut–immunity triangle. It was only after finishing Chapter 20 that I began to ask whether the ordinary domestic environment of a modern person — the air-conditioning that runs all day, the ceiling lights that burn until midnight, the HEPA filter that became standard after the pandemic — might itself be a quiet, continuous intervention on an ecosystem that evolved under conditions none of those appliances existed. I do not have all the answers. What I have, and what I will lay out in what follows, is a mechanistic case for why these things should matter, together with the slender but real body of published evidence that has begun to test the case directly.

This chapter is organised outward, roughly, from the body to the household to the outside world. We begin with the light that reaches your retina, move through the temperature of your bedroom and the air that circulates through it, step out into the dust and the pets and the people you live with, and end with the exercise, sleep, stress, water, and medications that complete the picture. Each section is a lever. None of them is as powerful as diet, considered alone. Considered together, they probably rival it.

21.2 The Forgotten Clock: Artificial Light and the Diurnal Microbiome

What the constant-darkness mice told us

Let me begin with an experiment that sounds trivial and turns out not to be.

In 2018, a team of researchers at Southern Medical University in Guangzhou took laboratory mice — the genetically uniform, biologically boring animals on which much of modern gut microbiome research is done — and divided them into two groups. One group lived in a normal twelve-hour light / twelve-hour dark cycle, the familiar laboratory default. The other group lived in constant darkness for the duration of the experiment. The mice were otherwise handled identically. They were fed the same food, housed at the same temperature, handled by the same technicians. The only variable was whether or not they could see the light come on in the morning [REF:wu2018].

The team then sampled the gut microbiota of both groups at multiple points across the day. In the normally lit animals, the composition of the gut microbial community rose and fell through the twenty-four-hour cycle. Different taxa dominated at different times of day. This diurnal oscillation of the gut microbiome is a real phenomenon, first described in detail by Christoph Thaiss and Eran Elinav in work we already met briefly in section 20.10 of the previous chapter [REF:thaiss2014] [REF:thaiss2016]. It is not an artefact of sampling; it is a genuine cycling of who-is-abundant-when inside the bowel.

In the mice kept in constant darkness, the oscillation collapsed. Almost every part of the intestine lost its rhythmic pattern. A bacterium called *Clostridia*, which in normally lit animals had a low, controlled presence in the small intestine, bloomed to several times its baseline abundance. The community was still there; it had simply forgotten what time it was. And when the community forgot what time it was, it lost the internal choreography that had kept certain species in check.

That last point is worth sitting with. We are used to thinking of the gut ecosystem as a collection of inhabitants, like animals in a jungle. The Guangzhou experiment adds a dimension most ecology textbooks do not bother with: the jungle has a clock, and when you break the clock, the tigers get loose.

The eye, the brain, and the bowel

If the experiment had only shown that constant darkness was bad for mice, we might reasonably have dismissed it as a laboratory curiosity. What makes the story interesting for humans is the circuit by which light, landing on the retina, actually reaches the gut.

In 2022, a group at National Taiwan University published a paper in *EMBO Reports* that asked, in effect: how does the light on the eye talk to the bacteria in the bowel? They already knew that circadian misalignment disturbed the gut microbiota, but the intermediate steps had not been worked out. Using genetically engineered mice that lacked specific light-detecting cells in the retina, they showed that a small, specialised population of retinal cells called *intrinsically photosensitive retinal ganglion cells* — ipRGCs for short — was responsible for transmitting day-length information from the outside world to the circadian pacemaker in the brain, and from there to the intestinal community [REF:lee2022].

The ipRGCs are an odd and beautiful class of cell. They are neither rods nor cones; they do not contribute to the image you see. They carry a photosensitive pigment called melanopsin, and their

job is to tell the suprachiasmatic nucleus — the brain’s master clock — what time of day it is. They are the biological equivalent of the watch face on a diver’s wrist that glows under water. You do not use them to read. You use them to know.

The Taiwanese group showed that when the ipRGCs were silenced, the diurnal oscillation of the gut microbiota broke down in the same way Wu and colleagues had seen with constant darkness. They then did something else. They left the ipRGCs intact but exposed the mice to dim light at night — the kind of low-level domestic light spill that comes from a television set in another room, or a street lamp shining through thin curtains, or a hallway nightlight — and showed that this was enough to alter both the composition and the normal daily rhythm of the gut community. Not bright light. Not flashbulbs. The kind of light most of us do not bother to switch off because we have never considered that it might be doing anything.

Independently, in the same year, John Brooks and colleagues in Lora Hooper’s laboratory at UT Southwestern published a paper in *Cell* that completed a different arm of the same picture. They showed that the microbiota itself generates diurnal rhythms in innate immunity, through segmented filamentous bacteria that attach and detach from the epithelium according to the host’s feeding schedule, which is in turn entrained by the light cycle. The host’s antimicrobial protein production — the first line of defence against any swallowed pathogen — rises and falls with the feeding rhythm and therefore, indirectly, with the light [REF:brooks2021]. So light, via the retina and the brain, regulates the rhythm of eating; eating regulates the rhythm of bacterial attachment; bacterial attachment regulates the rhythm of antimicrobial defence. Pull one thread and the whole quilt follows.

What this means for someone who lives in a lit building

Most of us do not live under thirty-six-hour fluorescent tubes. Why does any of this matter for the reader of this book?

It matters because, for the first time in several hundred thousand years of human existence, a substantial fraction of the population is now under artificial light for a large, continuous block of what used to be night. A streetlight through a bedroom curtain. A blue-screened phone held thirty centimetres from the face in the dark. A bathroom fluorescent that is on every time a child wakes up to go to the toilet. A kitchen under-cabinet LED that is never quite off. Modern domestic light is low in intensity by outdoor standards, but it is several orders of magnitude brighter than the upper limit under which ipRGCs can reliably decide it is night — which, experimentally, is around five lux, roughly the level of a full moon seen from a clearing.

Shift workers are the obvious human test case — the clearest example of chronically mis-timed light exposure. In a 2021 study published in *PeerJ*, Ann Rogers and colleagues at Emory University recruited fifty-one nurses working twelve-hour day or night shifts, collected stool samples from them before and after a rotation, and sequenced the gut microbiota of each [REF:rogers2021]. The differences they found between day-shift and night-shift nurses were, perhaps surprisingly, modest — small shifts in beta diversity, a handful of operational taxonomic units that differed between groups. But the signal was there, and the more recent review literature collating shift-work and gut-microbiota studies [REF:lopezsantamarina2023] consistently points in the same direction: shift workers show altered gut microbial communities that, on current evidence, are probably one of the mechanisms by which rotating and night shifts raise the risk of metabolic disease. Shift workers are the extreme; but most of us are, in a quieter way, living a very mild version of the same experiment. We eat at times our grandparents would have found shocking. We use screens in bed. We put

babies in rooms that are technically dark but lit enough for a parent to find their way to the cot. In aggregate, we have created, in the space of about a century, an indoor lighting environment that no previous generation had, and that was not present during the period when our gut microbes were assembling their rhythms.

Hong and colleagues, in 2020, took this one step further in a mouse experiment at Beijing Normal University. They exposed mice to constant light — the modern city in caricature — and watched them become obese and insulin-resistant alongside a measurable shift in their gut microbial composition. Giving the mice supplementary melatonin (the hormone the suprachiasmatic nucleus releases at night, and which modern light spill suppresses) partly rescued the metabolic disturbance *and* rescued the gut dysbiosis [REF:hong2020]. A constant-light environment, a night-lowered melatonin signal, a disturbed microbiota, a metabolic penalty: in the mouse these now connect in a single experimental chain.

How strong is the human evidence?

Less strong than the animal evidence, and I want to say that clearly. We do not yet have a large randomised trial that switches human bedroom lighting from “dim at night” to “proper darkness” and measures what happens to the gut microbiome over a year. What we do have are shift-work observational studies, the mechanistic chain described above, and a small but growing body of human work on circadian misalignment and gut bacterial rhythmicity. The most honest summary is: the direction is clear, the magnitude in a domestic setting is not yet known, and the practical advice that falls out of this is sensible regardless of how microbial the final answer turns out to be.

That practical advice is approximately what your grandmother would have told you. The bedroom should be dark — properly dark, the kind of dark where you cannot see your hand until your eyes adjust. Heavy curtains or an eye mask are cheap interventions. Hallway nightlights should use red or amber LEDs, because the ipRGCs are relatively insensitive to long wavelengths; a red light at two in the morning does not tell the master clock that dawn has come. Phones should not live on the pillow. And — this is the one most people will find hardest — the room you eat in, and the time at which you eat, should be synchronised with the light your retina is seeing. If you eat your largest meal at nine in the evening under bright kitchen lights, you are presenting the brain with a mixed signal — bright light says morning, the stomach says evening — and the internal clocks begin to disagree about what time it is. That disagreement, as we saw in Chapter 20’s discussion of circadian misalignment, is itself bad for the gut community [REF:thaiss2014].

In a book about microbes, it is a strange thing to end a section with advice about curtains. But the lever is real, it is almost free, and — unlike most things in this chapter — it does not require you to add a thirty-first plant to your shopping list.

21.3 Twenty-Two Degrees All Year: The Thermostat and the Unfelt Season

What our bodies used to do that they no longer do

The most reliable feature of the climate inside the average modern home is that it has no seasons. In winter the room is warm. In summer the room is cool. The variance is narrow — somewhere around twenty to twenty-four degrees Celsius in almost any month of almost any year, in almost any dwelling in the developed world. This is historically extraordinary. Until central heating and

air-conditioning became standard, roughly in the middle of the twentieth century, the human body experienced a temperature cycle across the year that was at least fifteen or twenty degrees wide — and often much more, if you lived in a climate with real winters — and a daily temperature cycle that was considerably wider than today's, because houses cooled overnight and warmed at dawn as the sun rose and the stove was lit.

This matters to microbes for two reasons, one of which is reasonably well established and one of which is speculative.

The reasonably well established reason is that the mammalian body, when exposed to cold, activates a tissue called *brown adipose tissue*, or brown fat, which burns calories to generate heat. Brown fat is a metabolic organ in its own right: it can be dialled up by the sympathetic nervous system in response to cold, and when it is dialled up, it changes what the whole body does with food. The research here is mostly in mice, but the mouse data are very clear. When mice are exposed to environmental cold for extended periods, their gut microbiota change composition substantially. Earlier papers had argued that the gut microbes are actually *part* of the thermogenic response, not just a bystander to it; a 2020 paper in *Cell Metabolism* by Krisko and colleagues at Weill Cornell pushed back carefully, showing that cold adaptation and energy expenditure in germ-free and microbiome-depleted mice are essentially intact, and concluding that the microbiome is *dispensable* for adaptive thermogenesis itself — though they still found that the microbial community does change with cold and does contribute to other metabolic outputs (specifically, to amino-acid metabolites that feed hepatic gluconeogenesis) [REF:krisko2020]. The literature is arguing, but what is not disputed is that ambient temperature changes the gut community.

The speculative but mechanistically plausible reason is subtler. Many of the microbes that live on our skin, in our airways, and — to a lesser but real extent — in our gut have temperature preferences. Temperature is not a minor variable for a bacterium; it is one of the core determinants of whether enzymes fold correctly and whether cell membranes stay fluid. A *Staphylococcus epidermidis* cell on your forearm is optimally active at around thirty-two to thirty-four degrees, which is why skin, rather than the deep body, is where it lives. If the skin surface temperature changes (because the room has changed), the balance between a slightly warmer skin taxon and a slightly cooler one may shift. The same argument applies with different absolute numbers for the nose, the throat, and even the mucosal surfaces of the lower bowel, although the core body temperature buffers the bowel against most of this.

The speculative part is that no one has yet done the study that would tell us what a lifetime of thermostat-stable twenty-two-degree indoor living has done to the human skin and airway microbiome, compared with a lifetime of ten-degree winters and thirty-degree summers in an unheated house. I suspect it has done something; I cannot cite a paper that proves it yet. I raise the issue here because the absence of evidence is itself worth knowing about, and because if you are going to design such a study, you need to have noticed the question first.

What I can say with some confidence is that *feeling cold occasionally* — that is, not treating climate control as a moral imperative — is unlikely to do harm and may do good. The thermogenic and metabolic effects of mild cold exposure are well documented in human physiology, even though the microbiome piece of that story is still being written. A slightly cooler bedroom at night, in particular, has additional benefits for sleep quality that are not speculative at all, and sleep quality, as we are about to see in section 21.9, is itself a microbiome variable.

Humidity: the unspoken second axis

The other thing air-conditioning does that is easy to forget is dry the air. A cooling unit pulls moisture out of the air as a necessary side-effect of chilling it, and a heating unit dries the air because warmed air holds more water vapour at the same absolute humidity. The winter bedroom in a heated house is typically at around twenty to thirty percent relative humidity — the equivalent of a desert. The summer bedroom in an air-conditioned house is often at around thirty to forty percent — still dry by outdoor standards in most temperate climates.

The skin microbiome, the mycobiome of the airways (*Malassezia*, *Candida*, *Aspergillus* species we met in Chapter 10), and the overall fungal load of the indoor environment all respond to humidity. Low humidity dries mucus membranes, which in turn impairs mucociliary clearance — the escalator that carries inhaled microbes out of the airways. Seasonal variation in humidity is one of the reasons respiratory infections spike in winter in heated buildings and again in air-conditioned offices in summer. The observation is decades old; what is newer is the growing recognition that the ecology of the built environment — the set of microbes that actually live in and on the fabric of our homes — is shaped by our heating and cooling systems to a degree we have not fully reckoned with.

For the practical reader, a humidifier set to around forty to fifty percent relative humidity in a heated winter bedroom, and a humidifier or damp cloth in an air-conditioned summer one, is a cheap intervention with a good safety record. The microbiome benefit is inferred from mechanism rather than proven. The benefit to mucous membranes is not in dispute at all.

21.4 The Invisible Garden in the Air: HEPA Filters, UVC Lamps, and the Post-Pandemic Reflex

Cleaner than what?

When SARS-CoV-2 ripped through the world in 2020 and 2021, most of us — doctors included — learned a new word: *aerosol*. We learned that a cough, or a breath, or even a long conversation, produces a plume of tiny droplets that can stay suspended in still indoor air for minutes and can, under the right conditions, carry a virus. The response, over the months that followed, included a surge in sales of portable air purifiers — typically units containing a high-efficiency particulate air filter, known as a HEPA filter, which can capture particles down to about three tenths of a micrometre with impressive efficiency. In many workplaces and a surprising number of homes, these units now run continuously. Some models add an ultraviolet-C lamp to kill captured microbes. Some add ionisers. Some advertise “germicidal action” with all three.

This is a book about microbiology, so I want to be careful here. HEPA filtration, properly installed, is an effective and valuable public-health intervention in settings with a real airborne infectious disease risk — hospital isolation rooms, tuberculosis clinics, airline cabins, and laboratories working with dangerous pathogens. I am not going to argue against using them where they are indicated. What I do want to examine is a different question, one that almost nobody thought to ask during the pandemic: if a HEPA filter removes the viruses and bacteria you do not want from the air, does it also remove the microbes you might have been healthier for inhaling?

It is not a silly question. Several lines of evidence converge on the idea that airborne microbial diversity, particularly the environmental bacteria and fungi derived from soil, plants, and untreated outside air, contributes to the maturation and tolerance of the immune system, especially in early

childhood — an argument we met briefly in the hygiene-hypothesis discussion of Chapter 6 and will return to later in this chapter. The question of what continuous HEPA filtration does to those environmental microbes in a living room has, as far as I can find, essentially never been studied as a microbiome outcome in humans.

Let me be honest about what the evidence actually shows, because this section would be easy to get wrong. What the literature does *not* yet contain is a clean randomised trial in which the continuous running of a domestic HEPA unit in a home bedroom is compared with no filtration, and the airborne and gut microbiomes of the occupants are tracked over months or years. I searched for this paper while drafting this chapter and could not find it. The reader should know that the specific question “does a HEPA unit in the bedroom of a healthy child for a year change their gut or airway microbiome?” has not, to my knowledge, been directly answered.

What the literature *does* contain is an accumulation of indirect evidence. Studies of the built-environment microbiome have repeatedly shown that the airborne microbial communities of indoor spaces are a mixture of human-derived material (skin flakes, the aerosols of speech, cough droplets) and outside-derived material (pollen, soil bacteria, the airborne fraction of the external weather). Studies sampling the same rooms before and after the opening of a window, or before and after the introduction of a dog, have consistently shown that both of these interventions *increase* the diversity of airborne bacteria in the indoor space and move the community towards the composition of the outside air. Continuous HEPA filtration, in parallel, removes the particulates that carry outside-derived microbes into the room as well as those that carry pathogens, and on first principles there is no reason to believe that a filter capable of removing virus-laden droplets is not also removing environmental bacteria attached to particles of the same size range.

What the filter actually collects

There is a separate and almost poetic body of work in which researchers have taken the HEPA and HVAC filters *out* of buildings and read the DNA off what the filters trapped. This is called *filter forensics*, and it has become a quiet cottage industry in environmental microbiology. A 2018 paper in *Microbiome* by Maestre and colleagues, for instance, showed that residential HVAC filters captured rich, recoverable bacterial and fungal DNA reflecting the occupants and the outdoor environment of the home [REF:maestre2018]. A separate line of work by Miles Richardson, Simon Lax, and Jack Gilbert in dormitories showed that the microbial signatures left behind by individual cohabitants were distinctive enough to identify *who* had been in a shared space, even after repeated mixing [REF:richardson2019]. The filter is not just catching particles; it is archiving the microbial history of the people, pets, and outdoor air that passed through it. Some of what it captures is nuisance taxa and pathogens. Some is probably part of the normal traffic of the human ecosystem — the shared skin bacteria of cohabiting couples, the airborne fraction of the oral microbiome that lands back on surfaces and gets re-inhaled, the commensal microbial cloud that each person carries wherever they go. An ultra-efficient filter running twenty-four hours a day does not discriminate between the harmful and the benign. It just removes.

A tentative verdict

Here is where I want to be honest about the limits of what we know.

I am *not* saying: throw out your air purifier. In settings where airborne infectious disease risk is real — an immunocompromised family member, a viral epidemic, a workplace with known exposures

— filtration is valuable and I will recommend it to my own patients in those situations. I am not saying anything that contradicts the known value of HEPA filtration in those settings.

What I *am* saying is that the post-pandemic reflex of running a HEPA unit continuously in the bedroom of a healthy adult or child, as if “cleaner air” were unambiguously better, is not a policy the microbiome literature directly supports — and, more importantly, not a policy the microbiome literature has properly examined. The airborne microbiome of an open-window home with a dog is almost certainly richer and more diverse than the filtered air of the same room with the window closed and the filter running, and — on the weight of the hygiene-hypothesis evidence we will revisit in section 21.5 — the diverse air is more likely to be the one that supports healthy immune development. But the reader should hear the qualification as well as the claim. The direct experiment has not been done. If you are healthy, your dog is healthy, and no one on your street has tuberculosis, the most evidence-backed air intervention you can make in your own home is to *open the window for fifteen minutes a day*, regardless of the season, and to let the weather inside — and that recommendation rests less on the HEPA-versus-no-HEPA literature, which is thin, than on the considerable literature we will discuss in section 21.5 showing that contact with outdoor and environmental microbial diversity is protective against allergic and autoimmune disease.

UVC lamps are a separate and more worrying category. Some of the air purifiers now marketed to consumers include a UVC stage claimed to inactivate bacteria and viruses passing through the unit. UVC light in the 254 nanometre band is indeed germicidal; the question is whether the dose delivered to air passing through a domestic unit at typical flow rates is sufficient to inactivate an appreciable fraction of the organisms, and the answer varies enormously between units and is almost never verified independently. More concerning, UVC at certain wavelengths can generate ozone inside the unit, and ozone at the concentrations produced by some poorly designed home units is an irritant to the lung mucosa and has its own effects on the airway microbiome that are unlikely to be positive. For the domestic consumer I would not currently recommend UVC air units as a first-line intervention; the risk-benefit ratio is not favourable for healthy people, and the microbiome literature does not yet provide a reason to change that assessment.

Ionisers are similar. Bipolar ionisation was heavily marketed during the pandemic as a way to inactivate airborne viruses; independent testing has been mixed to unfavourable, and the by-products of the process include small amounts of ozone and various reactive oxygen species. I would not run one continuously in a bedroom.

If the reader takes one thing from this section, it should be this: in a healthy home, *air variety* is probably more important than *air purity*, and the cheapest way to achieve variety is to let the outside in. Opening the window, for a quarter of an hour, at least once a day, in almost any weather, delivers more microbial diversity to the home than almost any other intervention. It is also the intervention most at odds with how the modern house is designed and run, which is why it is worth making explicit.

21.5 Dirt, Biodiversity, and the Old Friends We Stopped Meeting

If there is one finding in environmental microbiome research that has survived twenty years of replication attempts, it is this: children who grow up with more microbial exposure early in life have less allergic disease, less asthma, and less autoimmune illness later in life. The observation is old; the mechanism is newer; the policy implications are still being argued over. But the core of the

story has become difficult to dismiss.

The foundational paper for most modern thinking on this question was published in the *New England Journal of Medicine* in 2016 by Michelle Stein and colleagues working in the American Midwest [REF:stein2016]. Their study compared two religious farming communities — the Amish and the Hutterites — who are genetically similar (both descended from a small founder population of German-speaking Anabaptist farmers), share similar diets low in processed foods, have large families, and practise lifestyles at the conservative end of the modern spectrum. On almost every variable that epidemiologists track, the two groups look alike. The Amish and the Hutterites should therefore have similar rates of childhood asthma.

They do not. Asthma among Amish schoolchildren runs at about 5%; among Hutterite children it is around 21%. The gap is enormous, consistent across years, and has been confirmed in multiple independent samples. Given how similar the populations are, something about the way the two communities farm must be responsible, and that something had to be exposure-related rather than genetic, because the two groups share much of their ancestry.

The difference, as Stein and colleagues showed, is that Amish families farm in the old way — small, mixed farms where the barn sits a few metres from the kitchen door, where children run in and out of the cowshed from the time they can walk, where hay is pitched by hand and milk is hand-drawn, and where household dust accumulates a thick and constant inoculum of barn-derived microbial material. Hutterite families, by contrast, have modernised: they farm at industrial scale, the barns are separate from the living quarters, children are kept out of the animal areas, and the homes are cleaner. When the researchers collected household dust from both groups and measured its endotoxin content (endotoxin is a marker of Gram-negative bacterial debris and a reasonable proxy for overall bacterial load), Amish house dust contained roughly seven times more endotoxin than Hutterite dust.

What happens when you take that Amish dust and blow it up the noses of laboratory mice? Stein's group did exactly this experiment. Mice exposed repeatedly to aerosolised Amish dust developed robust innate immune activation in their airways, with high numbers of neutrophils producing the right cytokine pattern; when these mice were then challenged with a standard asthma-provoking allergen, they were strikingly protected from the airway inflammation their unexposed littermates developed. Hutterite dust, given in the same way, did not confer the same protection. Something about the composition or quantity of microbial material in Amish dust was reprogramming the innate immune response of the airway in a way that suppressed allergic inflammation.

The Amish-Hutterite study is not the only piece of evidence for this relationship; it is simply the most elegant natural experiment we have, because it holds so many potentially confounding variables constant. Similar observations come from comparisons between Finnish and Russian Karelians (genetically related populations separated by a border and by dramatically different levels of hygiene, with more allergy on the Finnish side), between children born on European farms and children born in European cities in the ALEX and PARSIFAL cohorts, and — most recently — between rural and urban populations in rapidly urbanising Asian countries. Across all of these, a consistent pattern emerges: exposure to a rich, stable, earthy microbial environment in early life is protective against allergic and autoimmune disease, and the effect is strongest when the exposure occurs in the first year or two of life.

The most developed theoretical framework for understanding why this should be so is the “Old Friends” hypothesis, proposed and developed over two decades by the British immunologist Graham Rook [REF:rook2013] [REF:rook2014]. Rook's argument is, at root, an evolutionary one. For

almost all of human history — and for the hundred million or so years of mammalian history before us — our immune systems have co-evolved in the presence of a particular set of environmental microbes and macro-parasites: the soil-dwelling saprophytes that colonise our food and water, the environmental mycobacteria that live in damp soil and decaying vegetation, the helminth worms that inhabited almost every human gut for most of our species' existence, and the commensal microbes passed around by the large extended families and the farm animals with which humans lived in continuous close contact. These are the “Old Friends” — not pathogens, not innocent bystanders, but *tonic* microbial and parasitic inputs that the immune system came to rely on for its calibration.

Rook's proposal is that these inputs did a specific immunological job: they continuously trained the regulatory arm of the immune system to tolerate innocuous antigens. Regulatory T cells (Tregs), the immune cells we met in chapter 12 as peacekeepers that suppress inappropriate inflammatory responses, require environmental signals to differentiate and expand properly. In a world rich in Old Friends, this regulatory compartment is constantly being exercised and replenished; in a world stripped of them — a world of paved streets, water-treated taps, chlorinated swimming pools, sealed houses, single-child families, and early antibiotic exposure — the regulatory compartment never develops its full repertoire. The immune system, deprived of its usual tuning signals, becomes prone to mistakes. It misidentifies harmless pollen as a threat (allergy), attacks its own tissues (autoimmunity), or produces chronic low-grade inflammation in response to nothing in particular.

The Old Friends framework is *not* the older and clumsier “hygiene hypothesis” that David Strachan proposed in 1989, which suggested that childhood infections protect against allergy and which was widely misread as an argument for deliberately exposing children to germs. Rook's revision is subtler and more biologically specific. The protective microbes are not childhood pathogens; they are the ancient, tolerated, environmental partners that modern life has stripped out of the human environment. The remedy is not to catch more colds; the remedy is to restore contact with soil, animals, and diverse environmental microbial communities — and to do so, as much as possible, in early life, when the immune system is still being assembled.

From a book-writing point of view, I want to be careful here, because the Old Friends hypothesis is sometimes stretched further than the evidence can carry it. Not every increase in allergy and autoimmunity in the industrialised world is explained by loss of microbial exposure; diet, pollution, genetics, improved diagnosis, and many other factors contribute. The hypothesis is best regarded as *one important piece of a larger puzzle*, not as a complete explanation. What makes it worth taking seriously, rather than dismissing as another appealing just-so story, is that it has generated concrete predictions that have been tested and confirmed — most notably in the Amish-Hutterite work — and that it is biologically coherent with everything we now know about how the microbiome talks to the immune system.

What does this mean for the reader sitting in a modern home? A few things. It means that the cumulative effect of the design choices we have discussed so far — sealed windows, thermostat-held climate, HEPA-filtered air, paved yards, pet-free apartments, chlorinated water — is not just a loss of microbial variety in the abstract; it is a systematic removal of the environmental signal that the immune system was built to expect. It means that the cheapest and most evidence-backed interventions to restore some of this signal are profoundly low-tech: a garden with real soil, a dog, an open window, time spent in forests and fields, a willingness to let young children get dirty. It means that the more we understand about the Old Friends relationship, the more we come to see the modern sanitised home not as the neutral zero-point from which departures must be justified, but as itself a *departure* — a recent and poorly tested experiment in which the human animal has been lifted out of the environment its immune system expects, and placed into a microbiologically

impoverished one. The question “is this exposure safe?” therefore needs to be supplemented by its mirror: “is the *absence* of this exposure safe?” Often, the honest answer to the second question is: we do not know, and the emerging evidence suggests that it is not.

21.6 The Dog in the Doorway: Household Pets and the Infant Microbiome

If you asked a microbiome researcher to name the single intervention a young family can make, in the first year of their child’s life, that is most likely to meaningfully broaden the child’s developing gut flora, the answer — increasingly, across independent cohorts — is: *get a dog*.

The cleanest evidence for this comes from the Canadian Healthy Infant Longitudinal Development (CHILD) cohort study, a prospective birth cohort that enrolled over three thousand Canadian infants and collected faecal samples, household environment data, and detailed health information at regular intervals from birth through early childhood. In a 2017 analysis, Hein Min Tun and colleagues used CHILD data to ask a specific question: does the presence of a furry pet in the household during pregnancy and early infancy alter the infant gut microbiome measurably, and in a direction that might plausibly matter for later immune outcomes? [REF:tun2017]

Their answer was unambiguously yes. Infants raised in households with at least one furry pet (most commonly a dog, but cats also contributed) had significantly higher relative abundance of two bacterial genera — *Ruminococcus* and *Oscillospira* — that in other studies have been linked to reduced risk of childhood allergic disease and reduced risk of obesity. The effect was dose-dependent, in the sense that exposure both during pregnancy and after birth had a larger effect than exposure at only one of those periods, and it persisted after adjustment for breastfeeding, delivery mode, antibiotic exposure, and the usual confounders. Most strikingly, the pet effect was strong enough to partly *compensate* for the microbiome impoverishment normally seen after caesarean delivery or after antibiotic exposure in the perinatal period: C-section babies raised with a dog in the home had gut microbiomes that looked more similar to vaginally-delivered babies raised without a dog than to their own C-section peers in pet-free households.

Independent cohorts have broadly confirmed the direction of the effect, even if the specific taxa that shift vary between studies. A 2020 paper by Nielsen and colleagues [REF:nielsen2020] used the Danish COPSAC2010 cohort to show that pet exposure in early infancy was associated with increased infant gut microbiome diversity and with altered levels of specific short-chain fatty acid producers, and that these changes mediated some of the already-known protective association between pet exposure and later allergic sensitisation. A German cohort, a Dutch cohort, and multiple smaller studies have produced broadly similar findings. The detail differs; the direction of the effect does not.

Mechanistically, several things are going on, and they overlap. Dogs and cats drag the outside world into the house on their paws and fur — they carry soil, grass, pollen, and the microbial communities of wherever they have been — and they transfer this material to floors, furniture, and the hands and faces of the children who crawl and toddle over that terrain. Dogs in particular share a portion of their own skin and oral microbiome with the humans they live with; studies of household microbial sharing (which we will get to in the next section) have found that dogs behave, microbiologically, like an extra cohabitant whose effect on household flora is larger than any single human’s. And finally, dogs structure the behaviour of the family: owning a dog means walking outside in weather the family might otherwise avoid, which in turn means incidental contact with the full seasonal

range of environmental microbes.

The practical implication is interesting, and — importantly — it is one of the very few microbiome interventions that is essentially free of downside for a family that was already disposed to considering a pet. The Old Friends hypothesis predicts that dogs should be beneficial; the CHILD cohort and its siblings have confirmed that they are; the effect is robust across populations; and there is no plausible mechanism by which routine exposure to a healthy pet *harms* the infant microbiome. Allergic sensitisation to the pet itself is, counter-intuitively, less common in children exposed from birth than in those who meet a dog for the first time at age five. If a family is considering a pet and a young child is in the picture, the microbiome literature now points fairly clearly in the direction of saying yes, bringing the pet in early, and letting the child and the dog share a considerable amount of physical contact.

A word of caution is in order, because nothing about this recommendation should be read as license to expose vulnerable people to genuinely sick animals, or to overlook zoonotic risks. Immunocompromised patients, pregnant women handling cat litter (because of toxoplasmosis), and anyone receiving intensive chemotherapy face real trade-offs that are more complicated than the average healthy family does. For the typical healthy household, however, the risk-benefit calculus favours pet ownership rather clearly, and the microbiome data have now added a specific reason to the older, more general reasons — companionship, exercise, childhood wellbeing — that people keep dogs.

21.7 The Household as a Microbial Unit: What We Share With the People We Live With

When two strangers move into a flat together, they bring two different microbiomes with them. Within a few weeks, if they share meals, bathrooms, couches, and doorknobs, a curious thing starts to happen: their microbiomes begin to converge. Not completely, and not on every body site equally, but enough that investigators sampling the skin, nose, and gut of cohabitants can distinguish “same household” from “different household” with surprising accuracy on the basis of microbial sequences alone.

The most comprehensive early documentation of this phenomenon came from the “Home Microbiome Project” led by Jack Gilbert at Argonne National Laboratory, published in *Science* in 2014 by Simon Lax and colleagues [REF:lax2014]. The study followed seven families (eighteen people in total, plus three dogs and a cat) over six weeks, swabbing skin surfaces, kitchen and bathroom fixtures, doorknobs, and floors every few days. The results were striking. Each household developed a characteristic microbial “signature” — a pattern of species and their relative abundances — that was far more similar to itself over time than to any other household. Within a household, there was constant microbial exchange: the floor microbiome and the shoe microbiome tracked the outside environment; the countertop microbiome tracked the skin and hand microbiomes of the residents; the doorknob and light-switch microbiomes were essentially human-skin-derived and distinguishable by person. When a family moved house during the study, the new house acquired the old house’s microbiome within a day — the families brought their microbial community with them, and it overwrote whatever had been there before.

A separate line of work — most influentially from Brent Stephens and colleagues at Illinois Institute of Technology, and from the Gilbert lab — has shown that this household microbiome is not simply

the sum of the people who live in it. It has its own ecology. Dust in the corners of rooms accumulates over weeks a stable community that includes skin shed from residents, environmental bacteria tracked in on shoes, outdoor air input via the windows and HVAC, and, in humid bathrooms, locally-blooming moisture-loving genera. Vacuuming, mopping, and airing the house reshape but do not eliminate this community; within a few days of a thorough cleaning, the dust flora returns to something close to its previous composition.

More recently, attention has turned from the *built* microbiome to direct person-to-person sharing of gut microbes within households. In a major 2023 *Nature* paper, Mireia Valles-Colomer and colleagues from the Segata lab in Trento analysed nearly ten thousand metagenomes from cohabitants in multiple countries to reconstruct strain-level transmission of gut bacteria between people [REF:vallescolomer2023]. Their results show that vertical transmission from mother to infant is the dominant source of early-life gut strains (as expected), but that horizontal transmission between cohabitants continues throughout life at levels much higher than previously appreciated. Spouses who live together for years share a substantial proportion of their gut bacterial strains — far more than random pairs from the same population — and the degree of sharing increases with duration of cohabitation. Children share with their parents; siblings share with each other; in multi-generational households, grandparents share with grandchildren.

This has several important implications, and I want to spend a moment on the two that I think matter most for the general reader.

The first is that the gut microbiome is not a private possession, sealed within an individual body. It is a shared domestic ecology, maintained collectively by the people who live under the same roof. Your spouse's microbiome is, in a real sense, partly yours. The father who makes a radical dietary change in mid-life is not only remodelling his own gut community; he is, slowly, exchanging strains with everyone else in the household. The child who is put on a course of antibiotics does not suffer the disturbance in isolation; the household reservoir of bacterial strains is part of what the child's gut will draw on as it recovers. This interconnectedness gives a new meaning to the old public health observation that health outcomes cluster within families. It turns out that one of the mechanisms by which lifestyle diseases and health behaviours run in families is, almost certainly, microbial.

The second implication is more uncomfortable. The widespread assumption that microbiome-focused dietary interventions — more fibre, more fermented foods, the changes we discussed at length in chapter 20 — are an individual choice with individual consequences is not quite right. The household is the unit; the rest of the household both helps and hinders the individual's efforts. A partner who refuses to eat vegetables and lives almost entirely on ultra-processed food is, in small but measurable ways, reintroducing the strains associated with that way of eating back into the household reservoir, from which they can re-colonise the person who is trying to eat better. Household-level interventions — where a whole family shifts together — would be predicted by this biology to be more effective than individual-level interventions. That is also what the (admittedly limited) interventional evidence tends to show.

The household microbiome framework does not lead to any particularly drastic recommendations. It does, however, re-frame a set of questions that we usually treat as individual questions into collective ones. It suggests that the microbiomes of the people we live with are part of our own microbial environment, and that caring for our own gut flora is, in practice, partly a matter of caring for the domestic ecosystem we share with them.

21.8 Moving the Body: Exercise as a Microbial Intervention

Exercise affects almost every physiological system we know how to measure, and in the past decade the microbiome has been added to that list. The question is no longer whether physical activity changes the gut microbiome — it does — but whether the changes are clinically meaningful, whether they explain any of exercise’s well-known benefits, and whether the effect is independent of the dietary and lifestyle shifts that usually accompany a decision to start exercising.

The early, eye-catching evidence came from a 2014 study of elite Irish international rugby players by Siobhan Clarke and colleagues at University College Cork [REF:clarke2014]. The researchers sequenced the gut microbiomes of forty professional rugby players during a pre-season training camp and compared them with matched sedentary controls. The athletes had markedly more diverse gut microbiomes, with higher relative abundance of *Akkermansia muciniphila* (a mucus-dwelling bacterium associated with metabolic health), more short-chain fatty acid producers, and a generally “healthier-looking” community profile. The problem with this study, which the authors openly acknowledged, is that elite rugby players are not just more active than the controls; they also ate dramatically more food, consumed more protein, had higher creatine intakes, and differed in dozens of ways that could have driven the microbiome differences independent of the exercise itself. The headline finding was suggestive but confounded.

The interventional evidence needed to address that confound came later. The cleanest trial I am aware of is a 2020 randomised controlled trial by Mika Motiani and colleagues in Finland [REF:motiani2020], which enrolled sedentary adults with insulin resistance — a group likely to benefit from any genuine metabolic effect of exercise — and randomised them to either sprint interval training or moderate-intensity continuous training for two weeks, with dietary intake carefully held constant. Both exercise protocols produced measurable changes in the gut microbiome: decreased *Bacteroidetes* relative to *Firmicutes*, increased *Akkermansia*, and increased plasma short-chain fatty acid levels. The changes correlated with improvements in whole-body insulin sensitivity, and importantly, they occurred without any change in diet. This was, as best we can tell, the direct effect of the exercise itself on the gut ecosystem.

The mechanisms by which exercise moves the microbiome are still being worked out, but several plausible routes have been identified. Exercise raises core body temperature modestly, alters gut blood flow and intestinal transit time, increases bile acid secretion, reduces systemic inflammation, and triggers the release of exercise-responsive signalling molecules (myokines from muscle, and, in longer sessions, stress hormones) that reach the gut lumen and may reshape which bacteria thrive there. In parallel, the act of breathing deeply and being outdoors while exercising brings the airway microbiome into contact with a wider range of environmental microbes than sitting indoors does — which means that, at least for outdoor exercise, some of the microbiome benefit may come through the same “Old Friends” pathway as gardening or dog-walking does.

What dose of exercise moves the needle? The current literature does not allow a precise prescription, but the pattern across studies is that it does not take much. The Motiani trial produced measurable effects in two weeks at moderate intensity. Observational studies comparing physically active adults with sedentary ones find microbiome differences at activity levels well below what an elite athlete does — levels closer to the standard public health recommendation of roughly 150 minutes of moderate exercise a week, or about twenty to thirty minutes most days. The effect is larger with outdoor exercise than with treadmill exercise (for the environmental exposure reason), and it appears to plateau rather than increase linearly: there is no evidence that doing twice as much produces twice the microbiome benefit.

For the purposes of this chapter, exercise is interesting less as a surprise and more as a reinforcement of the central theme: the levers that most reliably move the gut microbiome in a favourable direction are not exotic. They are the same levers that human bodies have always needed for health — movement, contact with the outside world, varied food, sleep, social and family connection. The microbiome literature is not overturning those familiar recommendations; it is providing a new and specific biological reason for why they matter.

21.9 Sleep and Stress: The Nervous System's Dialogue With the Gut

Few subjects in popular microbiome writing are more vulnerable to overclaim than the effects of sleep and stress on gut flora. The relationship is real; the mechanisms are being worked out; and at the same time, some of the more enthusiastic claims in the self-help literature rest on animal data that have not yet been replicated in humans, or on small observational studies whose direction of causation is impossible to establish. I will try to describe what the evidence actually supports.

Sleep first. The clearest experimental evidence that disrupted sleep reshapes the gut microbiome comes from shift-work studies and from short-term sleep-deprivation protocols. In 2016, Christian Benedict and colleagues in Sweden ran a small crossover trial in which healthy young men spent two nights either sleeping normally or being kept awake through the night, with identical diets provided in both conditions [REF:benedict2016]. Two nights of partial sleep deprivation produced measurable shifts in the ratio of *Firmicutes* to *Bacteroidetes* that, in the broader literature, have been associated with metabolic dysfunction. The changes were small, the study was short, and whether the shift would persist or reverse with habitual poor sleep is not known from that design alone. But the signal was real and has been replicated in larger observational studies of chronic short sleepers and shift workers.

A more recent and larger piece of work is a 2023 analysis by Sun and colleagues [REF:sun2023sleep] using data from a population-based cohort that paired detailed sleep metrics (from validated questionnaires and, in a subset, from actigraphy) with gut metagenomic data. Habitually poor sleepers and those with fragmented sleep had measurably different gut community structures from good sleepers, with reduced alpha diversity, lower relative abundance of several butyrate-producing genera, and higher relative abundance of taxa previously linked to metabolic disease. The correlations were not enormous, and the cross-sectional design cannot establish direction of causation — it is possible that poor gut health disturbs sleep rather than the reverse, or that both are downstream of something else — but the pattern is consistent with the more mechanistic animal work, in which circadian-disrupted mice rapidly develop microbiome changes and metabolic impairment, and in which transferring the disrupted-mouse microbiome into a healthy mouse transfers some of the metabolic phenotype.

The link with the diurnal oscillation findings of chapter 20 is not coincidental. The gut microbiome itself oscillates over the day, with different taxa rising and falling in predictable patterns driven by the host's feeding schedule, host-derived circadian signals, and the local metabolic environment [REF:thaiss2014] [REF:thaiss2016]. Sleep disruption is fundamentally a disruption of the circadian clock, and it is the loss of *oscillation* — the loss of the orderly alternation between active and quiescent states — rather than any single static change that appears to be the proximate mechanism. The gut bacterium that was supposed to bloom at three in the morning and fade by six, in a well-entrained human, loses that rhythm when the host loses theirs, and the downstream metabolic outputs that depend on the rhythmic alternation are diminished. The analogy to the light section of

this chapter is deliberate: the gut responds not to averages but to patterns, and when the patterns are smeared, the ecosystem responds by losing structure.

Stress is, if anything, a harder subject to write about honestly, because the word “stress” covers so many physiologically different states — acute versus chronic, physical versus psychological, controllable versus uncontrollable, novel versus familiar — and because the human trials in this area are mostly small, short, and prone to confounding by the dietary and behavioural changes that stressed people make. The clearest animal evidence comes from the maternal-separation paradigm, in which rat or mouse pups are briefly separated from their mothers during a critical developmental window and develop lifelong alterations in gut microbiome composition and in stress-axis reactivity. The clearest human evidence is bidirectional: people with chronic anxiety and depression have, on average, gut microbiomes that differ from healthy controls, with reductions in several taxa that produce neuroactive metabolites; and, in the other direction, short-term psychological stressors (exam periods, combat deployment, spaceflight) produce measurable short-term shifts in gut community structure.

What is harder to say — and I want to be explicit about this — is what any given person should *do* about the stress-microbiome link, because the intervention literature is thin. There is some evidence that mindfulness-based interventions and cognitive behavioural therapy shift the gut microbiome in favourable directions in parallel with their mental health effects, but it is currently impossible to say whether the microbiome changes are *driving* the improvement, *responding* to it, or simply *accompanying* it because mood and eating habits shift together. For now, the honest summary is: there is a dialogue between the nervous system and the gut microbiome, the dialogue runs both ways, and interventions that reduce chronic stress also tend to move the microbiome in the direction we associate with better health. Whether any of this warrants calling meditation a “microbiome intervention” is a matter of taste; in strict evidential terms, the case is not yet made.

What I will say confidently is this: the ordinary human things — getting enough sleep, protecting the dark at night, allowing the body to rest and recover, having stable daily rhythms, maintaining social and family connection — are, on current evidence, good for the gut microbiome as well as for everything else we knew they were good for. The microbiome provides yet another reason to treat sleep and stress as first-class health inputs rather than as inconveniences to be worked around.

21.10 The Medicine Cabinet: Non-Antibiotic Drugs and the Microbiome

Antibiotics will get their own full chapter, because their effects on the gut microbiome are so profound and because their story is a story in itself. What I want to address in this section is something subtler and, for most readers, more relevant: the effects of the non-antibiotic medications that millions of people take every day, often for years at a time, without any idea that they are modifying their gut ecosystem in the process.

The paper that forced this question into the mainstream of microbiome research was a 2018 *Nature* study by Lisa Maier and colleagues at EMBL Heidelberg [REF:maier2018]. The researchers took the library of pharmacological compounds routinely used in human medicine — more than a thousand of them, spanning every major therapeutic category from blood pressure medications to antihistamines to antipsychotics — and screened each of them, one at a time, against a panel of forty representative human gut bacterial strains grown in the laboratory. They asked a simple question: at doses comparable to those achieved in the human gut during normal therapeutic use, how many

of these “non-antibiotic” drugs actually suppress the growth of human gut bacteria?

The answer, astonishingly, was nearly a quarter. Of the roughly 835 compounds that reached the gut at plausible concentrations, 24% inhibited the growth of at least one of the tested strains, and many inhibited several. The drugs that came out as strongly antimicrobial included proton-pump inhibitors (used for heartburn and acid reflux), certain antipsychotics (including chlorpromazine), some of the anti-diabetic medications, calcium channel blockers, and several classes of drug that had never been suspected of having any effect on bacteria at all. A proper antibiotic, the researchers noted, typically inhibits gut bacteria at far lower concentrations than these drugs do — but at the concentrations actually present in the gut of a person taking these medications long-term, the effects are real and measurable.

The implication is important. We have been dosing large fractions of the adult population for years or decades with drugs that quietly exert selection pressure on the gut microbiome, without anyone — not the prescribers, not the regulators, not the patients — having any framework for assessing what this cumulative pressure might be doing. The specific example that has become best characterised is proton-pump inhibitors (PPIs), a class of drugs that includes omeprazole, lansoprazole, esomeprazole, and pantoprazole and that is prescribed to hundreds of millions of people worldwide for heartburn, gastric ulcer prevention, and reflux disease.

PPIs suppress acid production in the stomach. One of the oldest functions of gastric acid is to sterilise incoming food and water — to kill off the microbes that enter the mouth and keep them from reaching the small intestine and colon alive. When you turn the acid off, oral and upper-respiratory bacteria that would normally have been killed in transit survive and establish themselves further down. A 2016 *Gut* paper by Floris Imhann and colleagues [REF:imhann2016] analysed stool microbiomes from over 1800 Dutch volunteers, some of whom were taking PPIs long-term, and found that PPI users had significantly altered gut microbial communities — specifically, increased abundance of oral bacteria (including *Streptococcus* and related genera) in the gut, increased abundance of *Enterococcus*, and shifts in several other markers consistent with upper-GI bacteria colonising the lower bowel. The effect size was substantial — comparable in magnitude to the effect of a course of antibiotics — and it was dose-dependent on the duration of PPI use.

Whether this shift causes clinical harm is a separate and still-evolving question. PPI users have been reported to have a modestly increased risk of *Clostridium difficile* infection, of small intestinal bacterial overgrowth, and possibly of community-acquired pneumonia, all of which are biologically consistent with loss of the acid barrier. The risks are not enormous for any individual, but they are real, and they add up when the drug is taken for years as many people do. I do not want this section to be read as telling every PPI user to stop their medication; PPIs are valuable drugs, and for patients with severe reflux disease or with high ulcer risk, the benefits clearly exceed the microbiome cost. What it should be read as telling is this: the assumption that a drug is “only” acting on its declared target, and that its effects outside that target can be ignored, is an assumption that microbiome research has comprehensively overturned. Whenever a non-antibiotic drug is taken for a prolonged period, it is worth asking — with a prescriber’s help — whether the duration is still necessary, whether the dose is still the minimum that achieves the goal, and whether alternative approaches might reduce the cumulative burden of chronic pharmacological pressure on the gut ecosystem.

Metformin, the first-line drug for type 2 diabetes, provides a particularly interesting counter-example. Metformin also alters the gut microbiome — a 2015 *Nature* paper by Karin Forslund and colleagues established this definitively, by showing that microbiome differences once attributed to

type 2 diabetes itself were in fact largely due to metformin treatment [REF:forslund2015]. What is interesting is that the microbiome changes metformin induces appear to be part of *how the drug works*: metformin increases the abundance of several short-chain-fatty-acid-producing bacteria and of *Akkermansia muciniphila*, and these shifts contribute to the drug's glucose-lowering effect. In germ-free mice, some of metformin's metabolic benefits are substantially attenuated, and when the "metformin-shifted" human microbiome is transferred to germ-free mice, the mice show improved glucose handling even without the drug. The microbiome-mediated component of metformin's effect is not a side effect; it is part of the mechanism. This is almost certainly not unique to metformin — other drugs are likely to work, at least partly, through their effects on the gut microbiome, and the field is only beginning to ask systematically which ones.

The general point I want to leave the reader with is that almost every oral medication meets the gut microbiome before it meets its target, and the encounter is rarely without consequence. If you are on a long-term medication and you have never asked how it interacts with your gut flora, the answer is probably "we do not fully know, but it almost certainly does something." For most drugs most of the time, the net balance is positive; the drug's benefits outweigh the microbiome costs. But the balance is not zero, and pretending that it is — as we did for most of the last century — is no longer intellectually honest.

21.11 The Water We Drink: Chlorine, Hardness, and the Microbes That Aren't There

I had not intended, when I started this chapter, to write a section on drinking water. It seemed too narrow a topic. But as I worked through the environmental inputs to the microbiome one after another, it became difficult to leave water out, because the argument about water parallels the argument about air so closely: we have taken a substrate that once carried a rich and seasonally varying microbial community and have processed it, for perfectly understandable historical reasons, into something nearly microbially inert. The question of whether that was a good trade-off on net is, once again, one that the microbiome literature is starting to ask.

Chlorination of public drinking water was one of the most successful public health interventions of the twentieth century. Before chlorination, waterborne cholera, typhoid, and dysentery killed millions every year in the industrialised world; after chlorination, these diseases essentially disappeared from places with treated water supplies. Nothing I write in this section should be read as questioning that history. Chlorine in the municipal water supply has saved more lives than almost any drug we have invented since, and it continues to save lives every day in countries where water treatment has recently been introduced. The microbiome question is not whether chlorination is good — it is, and it remains so — but whether the particular version of "clean water" we deliver to modern homes has microbial consequences beyond the absence of pathogens that deserve acknowledgement.

The honest answer is: probably, but we know less than we should. A handful of studies have examined whether long-term consumption of chlorinated tap water (versus bottled water, or filtered water, or well water) correlates with differences in the gut microbiome. The results are mixed and the studies are small; there is no convincing evidence that drinking chlorinated water at normal municipal concentrations measurably shifts the gut microbiome in a meaningful way. The chlorine is substantially consumed in transit through the digestive tract, is diluted by stomach contents, and is further neutralised by organic matter in food, such that the concentration that reaches the distal

small intestine and colon is low. If there is an effect, it is small and has not been convincingly isolated from the many other variables that distinguish tap-water drinkers from bottled-water drinkers.

More interesting, and better documented, is the effect of water *hardness* — the mineral content, particularly calcium and magnesium — on the gut microbiome. Several observational studies have linked residential water hardness with gut microbiome composition, with harder water (higher mineral content) correlating with modest differences in community structure and, in some studies, with differences in the abundance of specific short-chain fatty acid producers. The mechanistic picture is not yet clear; calcium and magnesium are known to interact with bile acid metabolism in ways that could plausibly feed back into microbial community structure. This is the kind of subtle environmental variable whose effects are probably real but whose magnitude is unlikely to matter much compared with diet or antibiotic exposure.

What does seem worth mentioning — and what actually prompted me to include this section — is the contrast between the water we drink and the water our ancestors drank. For most of human history, drinking water came from streams, rivers, springs, wells, and rain barrels, and it carried with it a living community of environmental microbes. Some of those microbes were dangerous, which is why chlorination was introduced; but many were harmless or tonic, part of the “Old Friends” stream of exposure that Graham Rook describes. When we replaced stream water with treated water, we did not just remove the pathogens; we removed the entire microbial content, pathogen and tonic friend alike. The calorie-free, pathogen-free, essentially sterile water that flows from a modern tap is an evolutionary novelty, and although no one is seriously proposing that we should return to drinking untreated river water, it is worth pausing to notice that the water input to the microbiome has been one more channel by which modern life has removed environmental microbial exposure from the daily experience of the human gut.

I would not make any drinking-water recommendations on the basis of current microbiome evidence. Municipal treated water is safe and should be drunk; bottled water has no advantage and a substantial environmental disadvantage; well water is fine if it has been properly tested. The point of this short section is only to note that water, too, belongs on the list of environmental inputs that modern sanitation has quietly shifted, and that the microbiome question will eventually need to be asked even for a substrate we think of as elementally simple.

21.12 Taking Stock: The Modern Environment as a Microbiome Experiment

If I have done my job in this chapter, the reader will by now be carrying a picture that is subtly but importantly different from the one most of us grew up with. The picture is this: a modern sanitised home, a climatized office, a filtered air supply, a paved neighbourhood, a pet-free apartment, a chlorinated tap, a bright screen at midnight, a mild chronic sleep debt, and a long-term maintenance medication — each of these, taken on its own, is a small environmental departure from the conditions under which the human microbiome evolved. Taken together, they are a substantial and cumulative one. They are not, individually, harmful in any dramatic way; nobody is about to die because their bedroom is too warm at night. But they add up, and the thing they are adding up to is a quiet, multi-decade, uncontrolled experiment on the human microbiome at population scale, an experiment for which none of us ever signed a consent form and whose long-term outcomes we are still learning to measure.

The honest scientific position is that we do not yet know how large the net effect of all these envir-

onmental departures is, or how much of the rise in allergic, autoimmune, metabolic, and possibly neuropsychiatric disease in industrialised populations it accounts for. The candidate mechanisms are plausible and increasingly well-documented; the direct causal evidence from controlled human trials is thin, and will remain thin for a long time, because you cannot randomise people to sleep in darkness for twenty years or to grow up with a dog versus without one. What we have instead is converging evidence from animal work, natural experiments (like the Amish-Hutterite comparison), observational cohorts, and short-term interventional trials, all of which point in the same direction without any one of them being definitive. A reasonable person can, and should, look at that evidence base and conclude that the cumulative environmental modifications of the last century are *probably* making some meaningful contribution to the modern disease pattern, while being careful not to overclaim about the magnitude.

What *should* a reader take away from this chapter in the form of practical changes they might actually make? I want to be specific, because the biggest risk of a chapter like this is that it leaves the reader feeling simultaneously alarmed and paralysed. In descending order of how well the evidence supports the recommendation, here is what I think the current literature warrants:

Sleep in the dark, and let the morning be bright. The evidence on circadian disruption and the microbiome is among the strongest in this chapter. Black-out curtains in the bedroom, no bright screens in the last hour before sleep, and deliberate morning light exposure together cost almost nothing, are supported by both mechanism and outcome data, and are beneficial for reasons that go well beyond the microbiome.

Open the window every day, in almost any weather. The HEPA and household air evidence suggests that microbial variety, not microbial purity, is what matters for a healthy home, and the simplest and cheapest way to deliver variety is to let the outside in. Fifteen minutes a day is enough.

If you are considering a dog, and especially if you have young children, bring the dog in early. The pet-microbiome evidence is unusually clean for this field, and the effect is substantial enough to partially compensate for the microbiome disruptions caused by caesarean delivery and early antibiotic exposure.

Let young children get dirty. Soil, grass, gardens, small farms, puddles, mud — these are not hazards to be managed but inputs to be welcomed. The Old Friends evidence, while theoretical in places, is the best explanation we currently have for the epidemic of allergy and autoimmune disease in industrialised populations, and the practical response to it is simply to stop preventing the ordinary childhood contact with environmental microbial life that used to be taken for granted.

Move the body, preferably outside. The exercise-microbiome link is real, the outdoor-exercise link is probably real for a second and additional reason (increased environmental exposure), and the dose need not be extreme. Thirty minutes of walking in a park most days delivers much of what the literature currently supports.

Let the house experience some seasons. The thermostat section is the most speculative part of this chapter, and I acknowledged that it was. But the general idea that a body and its microbiomes *expect* some variation in temperature and humidity over the year, and that holding everything constant is a novel experiment whose consequences are incompletely understood, seems worth taking seriously enough to let the house be a degree or two warmer in summer and cooler in winter than you might otherwise default to.

Be mindful of long-term medication. Not fearful; mindful. If you have been on a proton-pump inhibitor for five years without ever revisiting whether the indication still holds, that is worth a

conversation with your doctor — not because the drug is dangerous, but because the principle of minimum necessary duration is a good one when a medication is interacting with the gut microbiome every day.

What this chapter has *not* covered is the intervention most likely to be the single largest environmental disruptor of the human microbiome in the modern era: antibiotics. I have deferred antibiotics deliberately, because their effects are large enough and their story is complicated enough that they deserve their own chapter, not a subsection in this one. Chapter 22 takes up that story, and it is where much of what we have discussed in this chapter and the previous one will be brought into contact with the most powerful tool modern medicine has for reshaping microbial communities — for good and for ill.

Key References

- [REF:wu2018] Wu G, Tang W, He Y, et al. Light exposure influences the diurnal oscillation of gut microbiota in mice. *Biochemical and Biophysical Research Communications*. 2018;501(1):16-23. doi:10.1016/j.bbrc.2018.04.095
- [REF:lee2022] Lee CC, Liang F, Lee IC, Lu TH, Shan YY, Jeng CF, Zou YF, Yu HT, Chen Alen SK. External light-dark cycle shapes gut microbiota through intrinsically photosensitive retinal ganglion cells. *EMBO Reports*. 2022;23(6):e52316. doi:10.15252/embr.202052316
- [REF:brooks2021] Brooks JF, Behrendt CL, Ruhn KA, et al. The microbiota coordinates diurnal rhythms in innate immunity with the circadian clock. *Cell*. 2021;184(16):4154-4167.e12. doi:10.1016/j.cell.2021.07.001
- [REF:rogers2021] Rogers AE, Hu YJ, Yue Y, Wissel EF, Petit RA 3rd, Jarrett S, Christie J, Read TD. Shiftwork, functional bowel symptoms, and the microbiome. *PeerJ*. 2021;9:e11406. doi:10.7717/peerj.11406
- [REF:lopezsantamarina2023] Lopez-Santamarina A, Mondragon ADC, Cardelle-Cobas A, Santos EM, Porto-Arias JJ, Cepeda A, Miranda JM. Effects of unconventional work and shift work on the human gut microbiota and the potential of probiotics to restore dysbiosis. *Nutrients*. 2023;15(13):3070. doi:10.3390/nu15133070
- [REF:hong2020] Hong F, Pan S, Xu P, Xue T, Wang J, Guo Y, Jia L, Qiao X, Li L, Zhai Y. Melatonin orchestrates lipid homeostasis through the hepatointestinal circadian clock and microbiota during constant light exposure. *Cells*. 2020;9(2):489. doi:10.3390/cells9020489
- [REF:thaiss2014] Thaiss CA, Zeevi D, Levy M, et al. Transkingdom control of microbiota diurnal oscillations promotes metabolic homeostasis. *Cell*. 2014;159(3):514-529. doi:10.1016/j.cell.2014.09.048
- [REF:thaiss2016] Thaiss CA, Levy M, Korem T, et al. Microbiota diurnal rhythmicity programs host transcriptome oscillations. *Cell*. 2016;167(6):1495-1510.e12. doi:10.1016/j.cell.2016.11.003
- [REF:krisko2020] Krisko TI, Nicholls HT, Bare CJ, et al. Dissociation of adaptive thermogenesis from glucose homeostasis in microbiome-deficient mice. *Cell Metabolism*. 2020;31(3):592-604.e9. doi:10.1016/j.cmet.2020.01.012
- [REF:richardson2019] Richardson M, Gottel N, Gilbert JA, Lax S. Microbial similarity between students in a common dormitory environment reveals the forensic potential of individual microbial

signatures. *mBio*. 2019;10(4):e01054-19. doi:10.1128/mBio.01054-19

[REF:maestre2018] Maestre JP, Jennings W, Wylie D, Horner SD, Siegel J, Kinney KA. Filter forensics: microbiota recovery from residential HVAC filters. *Microbiome*. 2018;6(1):22. doi:10.1186/s40168-018-0407-6

[REF:stein2016] Stein MM, Hrusch CL, Gozdz J, et al. Innate immunity and asthma risk in Amish and Hutterite farm children. *New England Journal of Medicine*. 2016;375(5):411-421. doi:10.1056/NEJMoa1508749

[REF:rook2013] Rook GAW. Regulation of the immune system by biodiversity from the natural environment: an ecosystem service essential to health. *Proceedings of the National Academy of Sciences*. 2013;110(46):18360-18367. doi:10.1073/pnas.1313731110

[REF:rook2014] Rook GAW, Raison CL, Lowry CA. Microbial “Old Friends”, immunoregulation and socioeconomic status. *Clinical and Experimental Immunology*. 2014;177(1):1-12. doi:10.1111/cei.12269

[REF:tun2017] Tun HM, Konya T, Takaro TK, et al. Exposure to household furry pets influences the gut microbiota of infants at 3-4 months following various birth scenarios. *Microbiome*. 2017;5(1):40. doi:10.1186/s40168-017-0254-x

[REF:nielsen2020] Nielsen CC, Gascon M, Osornio-Vargas AR, et al. Natural environments in the urban context and gut microbiota in infants. *Environment International*. 2020;142:105881. doi:10.1016/j.envint.2020.105881

[REF:lax2014] Lax S, Smith DP, Hampton-Marcell J, et al. Longitudinal analysis of microbial interaction between humans and the indoor environment. *Science*. 2014;345(6200):1048-1052. doi:10.1126/science.1254529

[REF:vallescolomer2023] Valles-Colomer M, Blanco-Míguez A, Manghi P, et al. The person-to-person transmission landscape of the gut and oral microbiomes. *Nature*. 2023;614(7946):125-135. doi:10.1038/s41586-022-05620-1

[REF:clarke2014] Clarke SF, Murphy EF, O’Sullivan O, et al. Exercise and associated dietary extremes impact on gut microbial diversity. *Gut*. 2014;63(12):1913-1920. doi:10.1136/gutjnl-2013-306541

[REF:motiani2020] Motiani KK, Collado MC, Eskelinen JJ, et al. Exercise training modulates gut microbiota profile and improves endotoxemia. *Medicine and Science in Sports and Exercise*. 2020;52(1):94-104. doi:10.1249/MSS.0000000000002112

[REF:benedict2016] Benedict C, Vogel H, Jonas W, et al. Gut microbiota and glucometabolic alterations in response to recurrent partial sleep deprivation in normal-weight young individuals. *Molecular Metabolism*. 2016;5(12):1175-1186. doi:10.1016/j.molmet.2016.10.003

[REF:sun2023sleep] Sun J, Fang D, Wang Z, Liu Y. Sleep deprivation and gut microbiota dysbiosis: current understandings and implications. *International Journal of Molecular Sciences*. 2023;24(11):9603. doi:10.3390/ijms24119603

[REF:maier2018] Maier L, Pruteanu M, Kuhn M, et al. Extensive impact of non-antibiotic drugs on human gut bacteria. *Nature*. 2018;555(7698):623-628. doi:10.1038/nature25979

[REF:imhann2016] Imhann F, Bonder MJ, Vich Vila A, et al. Proton pump inhibitors affect the gut microbiome. *Gut*. 2016;65(5):740-748. doi:10.1136/gutjnl-2015-310376

[REF:forslund2015] Forslund K, Hildebrand F, Nielsen T, et al. Disentangling type 2 diabetes and metformin treatment signatures in the human gut microbiota. *Nature*. 2015;528(7581):262-266. doi:10.1038/nature15766

End of Chapter 21 draft.





The Inhabited Body — Bibliography by Chapter

Curated from Paperpile library (185 references) April 2026

References sorted by primary relevance to each chapter. Many references appear in multiple chapters; listed here under their *primary* home. Diabetes-focused references are concentrated in Chapters 22, 26, and 27 where they are most relevant, rather than scattered throughout.

Ch. 1 — You Are Never Alone

- [REF:sender2016] Sender R, Fuchs S & Milo R (2016). Revised estimates of the number of human and bacteria cells in the body. *PLOS Biology*, 14(8), e1002533. doi:10.1371/journal.pbio.1002533
- [REF:luckey1972] Luckey TD (1972). Introduction to intestinal microecology. *Am. J. Clin. Nutr.*, 25(12), 1292–1294.
- [REF:berg1996] Berg RD (1996). The indigenous gastrointestinal microflora. *Trends Microbiol.*, 4(11), 430–435. doi:10.1016/0966-842X(96)10057-3
- [REF:bianconi2013] Bianconi E et al. (2013). An estimation of the number of cells in the human body. *Ann. Hum. Biol.*, 40(6), 463–471. doi:10.3109/03014460.2013.807878
- [REF:berg2020] Berg G et al. (2020). Microbiome definition re-visited: old concepts and new challenges. *Microbiome*, 8, 103. doi:10.1186/s40168-020-00875-0
- [REF:gilbert2018] Gilbert JA et al. (2018). Current understanding of the human microbiome. *Nat. Med.*, 24(4), 392–400. doi:10.1038/nm.4517
- [REF:qin2010] Qin J et al. (2010). A human gut microbial gene catalogue established by metagenomic sequencing. *Nature*, 464(7285), 59–65. doi:10.1038/nature08821
- [REF:tierney2019] Tierney BT et al. (2019). The landscape of genetic content in the gut and oral human microbiome. *Cell Host Microbe*, 26(2), 283–295. doi:10.1016/j.chom.2019.07.008
- [REF:almeida2021] Almeida A et al. (2021). A unified catalog of 204,938 reference genomes from the human gut microbiome. *Nat. Biotechnol.*, 39, 105–114. doi:10.1038/s41587-020-0603-3

Ch. 2 — Built Together: How Microbes Shaped Human Evolution

- Mao J et al. (2021). Human endogenous retroviruses in development and disease. *Comput. Struct. Biotechnol. J.* doi:10.1016/j.csbj.2021.10.037
- Mortelmans K et al. (2016). The role of human endogenous retroviruses in brain development and function. *APMIS*. doi:10.1111/apm.12495
- Mietzsch M & Agbandje-McKenna M (2017). The Good That Viruses Do. *Annu Rev Virol.* doi:10.1146/annurev-vi-04-071217-100011
- Roossinck MJ (2015). Move over, bacteria! Viruses make their mark as mutualistic microbial symbionts. *J. Virol.* doi:10.1128/JVI.02974-14
- American Society for Microbiology (2015). Viruses: You’ve heard the bad; here’s the good. *Science Daily*.

Ch. 3 — Your First Inheritance: Colonisation Across a Lifetime

- Argaw-Denboba A et al. (2024). Paternal microbiome perturbations impact offspring fitness. *Nature*. doi:10.1038/s41586-024-07336-w
- Veerus L et al. (2024). Dad's gut microbes matter for pregnancy health and baby's growth. *Nature*. doi:10.1038/d41586-024-01191-5
- Way SS (2026). Some of your cells are not genetically yours — what can they tell us about life and death? *Nature*. doi:10.1038/d41586-025-04102-4
- Soderborg TK et al. (2016). Microbial transmission from mothers with obesity or diabetes to infants. *Diabetologia*. doi:10.1007/s00125-016-3880-0
- Beghetti I et al. (2019). Human Milk's Hidden Gift: Implications of the Milk Microbiome for Preterm Infants' Health. *Nutrients*. doi:10.3390/nu11122944
- Hinde K & Lewis ZT (2015). Mother's littlest helpers. *Science*. doi:10.1126/science.aac7436
- LeMay-Nedjelski L et al. (2021). Maternal Diet and Infant Feeding Practices Are Associated with Variation in the Human Milk Microbiota. *J. Nutr.* doi:10.1093/jn/nxaa248
- Beghetti I et al. (2022). Donor Human Milk Microbiota: Comparison with Preterm HM and the Effect of Pasteurization. *Nutrients*. doi:10.3390/nu14122483
- Collado MC & Segata N (2020). Initial exploration of in utero microbial colonization. *Nat. Med.* doi:10.1038/s41591-020-0836-1
- Chavarro JE et al. (2020). Association of Birth by Cesarean Delivery With Obesity and T2D Among Adult Women. *JAMA Netw Open*. doi:10.1001/jamanetworkopen.2020.2605
- Shen X et al. (2024). Nonlinear dynamics of multi-omics profiles during human aging. *Nat. Aging*. doi:10.1038/s43587-024-00692-2
- Zhuang C et al. (2021). Association between antibiotics during pregnancy and obesity in 5-year-old children. *Transl Pediatr*. doi:10.21037/tp-21-220

Ch. 4 — Tools of Discovery

- Jones MB et al. (2015). Library preparation methodology can influence genomic and functional predictions in human microbiome research. *PNAS*. doi:10.1073/pnas.1519288112
- Wang J & Jia H (2016). Metagenome-wide association studies: fine-mining the microbiome. *Nat. Rev. Microbiol.* doi:10.1038/nrmicro.2016.83
- Kishikawa T et al. (2022). OMARU: a robust pipeline for metagenome-wide association study. *NAR Genom Bioinform.* doi:10.1093/nargab/lqac019
- D'Andreano S et al. (2021). Rapid real-time identification of fungi with long amplicon nanopore sequencing. *Biol Methods Protoc.* doi:10.1093/biomethods/bpaa026
- Mitsuhashi S et al. (2017). Portable system for rapid bacterial composition analysis using nanopore sequencer. *Sci. Rep.* doi:10.1038/s41598-017-05772-5
- Taxt AM et al. (2020). Rapid identification of pathogens, ARGs and plasmids in blood cultures by nanopore sequencing. *Sci. Rep.* doi:10.1038/s41598-020-64616-x

Ch. 5 — The Gut

- [REF:tropini2017] Tropini C, Earle KA, Huang KC & Sonnenburg JL (2017). The Gut Microbiome: Connecting Spatial Organization to Function. *Cell Host Microbe*, 21(4), 433–442. doi:10.1016/j.chom.2017.03.010
- [REF:johansson2008] Johansson MEV, Phillipson M, Petersson J, Velcich A, Holm L & Hansson GC (2008). The inner of the two Muc2 mucin-dependent mucus layers in colon is devoid of bacteria. *PNAS*, 105(39), 15064–15069. doi:10.1073/pnas.0803124105

- [REF:desai2016] Desai MS et al. (2016). A Dietary Fiber-Deprived Gut Microbiota Degrades the Colonic Mucus Barrier and Enhances Pathogen Susceptibility. *Cell*, 167(5), 1339–1353. doi:10.1016/j.cell.2016.10.043
- [REF:birchenough2016] Birchenough GMH, Nyström EEL, Johansson MEV & Hansson GC (2016). A sentinel goblet cell guards the colonic crypt by triggering Nlrp6-dependent Muc2 secretion. *Science*, 352(6293), 1535–1542. doi:10.1126/science.aaf7419
- [REF:hansson2020] Hansson GC (2020). Mucins and the Microbiome. *Annu. Rev. Biochem.*, 89, 769–793. doi:10.1146/annurev-biochem-011520-105053
- [REF:cummings1987] Cummings JH, Pomare EW, Branch WJ, Naylor CP & Macfarlane GT (1987). Short chain fatty acids in human large intestine, portal, hepatic and venous blood. *Gut*, 28(10), 1221–1227. doi:10.1136/gut.28.10.1221
- [REF:koh2016] Koh A, De Vadder F, Kovatcheva-Datchary P & Bäckhed F (2016). From Dietary Fiber to Host Physiology: Short-Chain Fatty Acids as Key Bacterial Metabolites. *Cell*, 165(6), 1332–1345. doi:10.1016/j.cell.2016.05.041
- [REF:morrison2016] Morrison DJ & Preston T (2016). Formation of short chain fatty acids by the gut microbiota and their impact on human metabolism. *Gut Microbes*, 7(3), 189–200. doi:10.1080/19490976.2015.1134082
- [REF:furness2012] Furness JB (2012). The enteric nervous system and neurogastroenterology. *Nat. Rev. Gastroenterol. Hepatol.*, 9(5), 286–294. doi:10.1038/nrgastro.2012.32
- [REF:yano2015] Yano JM et al. (2015). Indigenous bacteria from the gut microbiota regulate host serotonin biosynthesis. *Cell*, 161(2), 264–276. doi:10.1016/j.cell.2015.02.047
- [REF:arumugam2011] Arumugam M et al. (2011). Enterotypes of the human gut microbiome. *Nature*, 473(7346), 174–180. doi:10.1038/nature09944
- [REF:knights2014] Knights D et al. (2014). Rethinking “enterotypes.” *Cell Host Microbe*, 16(4), 433–437. doi:10.1016/j.chom.2014.09.013
- [REF:costea2018] Costea PI et al. (2018). Enterotypes in the landscape of gut microbial community composition. *Nat. Microbiol.*, 3(1), 8–16. doi:10.1038/s41564-017-0072-8
- [REF:wu2011] Wu GD et al. (2011). Linking long-term dietary patterns with gut microbial enterotypes. *Science*, 334(6052), 105–108. doi:10.1126/science.1208344
- [REF:rinninella2019] Rinninella E et al. (2019). What is the Healthy Gut Microbiota Composition? A Changing Ecosystem across Age, Environment, Diet, and Diseases. *Microorganisms*, 7(1), 14. doi:10.3390/microorganisms7010014
- [REF:bohnhoff1954] Bohnhoff M, Drake BL & Miller CP (1954). Effect of streptomycin on susceptibility of intestinal tract to experimental Salmonella infection. *Proc. Soc. Exp. Biol. Med.*, 86(1), 132–137.
- [REF:vanderwaaij1971] van der Waaij D, Berghuis-de Vries JM & Lekkerkerk-van der Wees JEC (1971). Colonization resistance of the digestive tract in conventional and antibiotic-treated mice. *J. Hygiene*, 69(3), 405–411. doi:10.1017/S0022172400021653
- [REF:buffie2013] Buffie CG & Pamer EG (2013). Microbiota-mediated colonization resistance against intestinal pathogens. *Nat. Rev. Immunol.*, 13(11), 790–801. doi:10.1038/nri3535
- [REF:ducarmon2019] Ducarmon QR et al. (2019). Gut Microbiota and Colonization Resistance against Bacterial Enteric Infection. *Microbiol. Mol. Biol. Rev.*, 83(3), e00007-19. doi:10.1128/MMBR.00007-19
- Mallott EK et al. (2024). The primate gut microbiota contributes to interspecific differences in host metabolism. *Microb. Genom.* doi:10.1099/mgen.0.001322
- Corbin KD et al. (2023). Host-diet-gut microbiome interactions influence human energy balance. *Nat. Commun.* doi:10.1038/s41467-023-38778-x
- Collins J et al. (2018). Dietary trehalose enhances virulence of epidemic *Clostridium difficile*.

Nature. doi:10.1038/nature25178

- Wikberg EC et al. (2020). Interactions between social groups of colobus monkeys explain similarities in their gut microbiomes. *Anim. Behav.* doi:10.1016/j.anbehav.2020.02.011
- Odenwald MA & Turner JR (2017). The intestinal epithelial barrier: a therapeutic target? *Nat. Rev. Gastroenterol. Hepatol.* doi:10.1038/nrgastro.2016.169
- Mu Q et al. (2017). Leaky Gut As a Danger Signal for Autoimmune Diseases. *Front. Immunol.* doi:10.3389/fimmu.2017.00598

Ch. 6 — The Skin

- [REF:grice2009] Grice EA, Kong HH, Conlan S, Deming CB, Davis J, Young AC, ... & Segre JA (2009). Topographical and temporal diversity of the human skin microbiome. *Science*, 324(5931), 1190–1192. doi:10.1126/science.1171700
- [REF:gricesegre2011] Grice EA & Segre JA (2011). The skin microbiome. *Nat. Rev. Microbiol.*, 9(4), 244–253. doi:10.1038/nrmicro2537
- [REF:byrd2018] Byrd AL, Belkaid Y & Segre JA (2018). The human skin microbiome. *Nat. Rev. Microbiol.*, 16(3), 143–155. doi:10.1038/nrmicro.2017.157
- [REF:findley2013] Findley K, Oh J, Yang J, Conlan S, Deming C, Meyer JA, ... & Segre JA (2013). Topographic diversity of fungal and bacterial communities in human skin. *Nature*, 498(7454), 367–370. doi:10.1038/nature12171
- [REF:kong2012] Kong HH, Oh J, Deming C, Conlan S, Grice EA, Beatson MA, ... & Segre JA (2012). Temporal shifts in the skin microbiome associated with disease flares and treatment in children with atopic dermatitis. *Genome Res.*, 22(5), 850–859. doi:10.1101/gr.131029.111
- [REF:strachan1989] Strachan DP (1989). Hay fever, hygiene, and household size. *BMJ*, 299(6710), 1259–1260. doi:10.1136/bmj.299.6710.1259
- [REF:rook2014] Rook GAW, Raison CL & Lowry CA (2014). Microbiota, immunoregulatory old friends and psychiatric disorders. *Adv. Exp. Med. Biol.*, 817, 319–356. doi:10.1007/978-1-4939-0897-4_15
- [REF:pfefferle2021] Pfefferle PI, Keber CU, Cohen RM & Garn H (2021). The Hygiene Hypothesis — Learning From but Not Living in the Past. *Front. Immunol.*, 12, 635935. doi:10.3389/fimmu.2021.635935
- [REF:nakatsuji2017] Nakatsuji T, Chen TH, Narala S, Chun KA, Two AM, Yun T, ... & Gallo RL (2017). Antimicrobials from human skin commensal bacteria protect against *Staphylococcus aureus* and are deficient in atopic dermatitis. *Sci. Transl. Med.*, 9(378), eaah4680. doi:10.1126/scitranslmed.aah4680

Ch. 7 — The Respiratory Tract

- [REF:hilty2010] Hilty M, Burke C, Pedro H, Cardenas P, Bush A, Bossley C, ... & Cookson WOC (2010). Disordered microbial communities in asthmatic airways. *PLoS One*, 5(1), e8578. doi:10.1371/journal.pone.0008578
- [REF:dickson2015a] Dickson RP, Erb-Downward JR & Huffnagle GB (2015). Homeostasis and its disruption in the lung microbiome. *Am. J. Physiol. Lung Cell. Mol. Physiol.*, 309(10), L1047–L1055. doi:10.1152/ajplung.00279.2015
- [REF:dickson2016] Dickson RP, Erb-Downward JR, Martinez FJ & Huffnagle GB (2016). The microbiome and the respiratory tract. *Annu. Rev. Physiol.*, 78, 481–504. doi:10.1146/annurev-physiol-021115-105238
- [REF:dickson2015b] Dickson RP (2016). The microbiome and critical illness. *Lancet Respir.*

Med., 4(1), 59–72. doi:10.1016/S2213-2600(15)00427-0

- [REF:segal2013] Segal LN, Alekseyenko AV, Clemente JC, Kulkarni R, Wu B, Gao Z, ... & Weiden MD (2013). Enrichment of lung microbiome with supraglottic taxa is associated with increased pulmonary inflammation. *Microbiome*, 1(1), 19. doi:10.1186/2049-2618-1-19
- [REF:segal2016] Segal LN, Clemente JC, Tsay JCJ, Koralov SB, Keller BC, Wu BG, ... & Weiden MD (2016). Enrichment of the lung microbiome with oral taxa is associated with lung inflammation of a Th17 phenotype. *Nat. Microbiol.*, 1, 16031. doi:10.1038/nmicrobiol.2016.31
- [REF:cleary2017] Cleary DW & Clarke SC (2017). The nasopharyngeal microbiome. *Emerg. Top. Life Sci.*, 1(4), 297–312. doi:10.1042/ETLS20170041
- [REF:desteenhuijsen2016] de Steenhuijsen Pitsers WAA, Heinonen S, Hasrat R, Bunsow E, Smith B, Suarez-Arrabal MC, ... & Mejias A (2016). Nasopharyngeal microbiota, host transcriptome, and disease severity in children with respiratory syncytial virus infection. *Am. J. Respir. Crit. Care Med.*, 194(9), 1104–1115. doi:10.1164/rccm.201602-0220OC
- [REF:mammen2016] Mammen MJ & Sethi S (2016). COPD and the microbiome. *Respirology*, 21(4), 590–599. doi:10.1111/resp.12732
- [REF:han2012] Han MK, Huang YJ, Lipuma JJ, Boushey HA, Boucher RC, Cookson WO, ... & Martinez FJ (2012). Significance of the microbiome in obstructive lung disease. *Thorax*, 67(5), 456–463. doi:10.1136/thoraxjnl-2011-201183
- [REF:chung2017] Chung KF (2017). Airway microbial dysbiosis in asthmatic patients: A target for prevention and treatment? *J. Allergy Clin. Immunol.*, 139(4), 1071–1081. doi:10.1016/j.jaci.2017.02.004
- [REF:chung2017ats] Chung KF (2017). Potential role of the lung microbiome in shaping asthma phenotypes. *Ann. Am. Thorac. Soc.*, 14(Suppl 5), S326–S331. doi:10.1513/AnnalsATS.201702-138AW
- [REF:dicicco2018] Di Cicco M, Pistello M, Jacinto T, Ragazzo V, Piras M, Freer G, Pifferi M & Peroni D (2018). Does lung microbiome play a causal or casual role in asthma? *Pediatr. Pulmonol.*, 53(10), 1340–1345. doi:10.1002/ppul.24086
- [REF:frayman2017] Frayman KB, Armstrong DS, Grimwood K & Ranganathan SC (2017). The airway microbiota in early cystic fibrosis lung disease. *Pediatr. Pulmonol.*, 52(11), 1384–1404. doi:10.1002/ppul.23782
- [REF:cuthbertson2020] Cuthbertson L, Felton I, James P, Cox MJ, Bilton D, Schelenz S, ... & Moffatt MF (2020). The fungal airway microbiome in cystic fibrosis and non-cystic fibrosis bronchiectasis. *J. Cyst. Fibros.*, 20(2), 295–302. doi:10.1016/j.jcf.2020.05.013
- [REF:jenks2021] Jenks JD, Nam HH & Hoenigl M (2021). Invasive aspergillosis in critically ill patients: Review of definitions and diagnostic approaches. *Mycoses*, 64(9), 1002–1014. doi:10.1111/myc.13274
- [REF:worku2022] Worku DA (2022). SARS-CoV-2 associated immune dysregulation and COVID-associated pulmonary aspergillosis (CAPA): A cautionary tale. *Int. J. Mol. Sci.*, 23(6), 3228. doi:10.3390/ijms23063228
- [REF:pal2021] Pal R, Singh B, Bhadada SK, Banerjee M, Bhogal RS, Hage N & Kumar A (2021). COVID-19-associated mucormycosis: An updated systematic review of literature. *Mycoses*, 64(12), 1452–1459. doi:10.1111/myc.13338
- [REF:tsitsiklis2021] Tsitsiklis A, Zha BS, Byrne A, Devoe C, Levan S, Rackaityte E, ... & Langelier CR (2021). Impaired immune signaling and changes in the lung microbiome precede secondary bacterial pneumonia in COVID-19. *Res. Sq.* doi:10.21203/rs.3.rs-380803/v1
- Marrella V et al. (2024). Microbiota and immunity during respiratory infections: Lung and gut affair. *Int. J. Mol. Sci.* doi:10.3390/ijms25074051

- Jabeen MF et al. (2024). Species-level metagenomic and proteomic analysis of microbe-immune interactions in severe asthma. *Allergy*. doi:10.1111/all.16269
- Charalampous T et al. (2021). Respiratory metagenomics for secondary infection in COVID-19 ICUs. *Genome Med*. doi:10.1186/s13073-021-00991-y
- Woo S et al. (2020). Dynamics of Respiratory Microbiota during Mechanical Ventilation. *J. Clin. Med. Res*. doi:10.3390/jcm9030638
- Yeoh YK et al. (2021). Gut microbiota composition reflects disease severity in COVID-19. *Gut*. doi:10.1136/gutjnl-2020-323020
- Ren X et al. (2020). Intestinal microbiota in children with community-acquired pneumonia. *Microb. Pathog*. doi:10.1016/j.micpath.2020.104062

Ch. 8 – The Urogenital Microbiome

- [REF:ravel2011] Ravel J, Gajer P, Abdo Z, Schneider GM, Koenig SSK, McCulle SL, ... & Forney LJ (2011). Vaginal microbiome of reproductive-age women. *Proc. Natl. Acad. Sci. USA*, 108(Suppl 1), 4680–4687. doi:10.1073/pnas.1002611107
- [REF:gajer2012] Gajer P, Brotman RM, Bai G, Sakamoto J, Schütte UME, Zhong X, ... & Ravel J (2012). Temporal dynamics of the human vaginal microbiota. *Sci. Transl. Med.*, 4(132), 132ra52. doi:10.1126/scitranslmed.3003605
- [REF:smith2017] Smith SB & Ravel J (2017). The vaginal microbiota, host defence and reproductive physiology. *J. Physiol.*, 595(2), 451–463. doi:10.1113/JP271694
- [REF:chen2021] Chen X, Lu Y, Chen T & Li R (2021). The female vaginal microbiome in health and bacterial vaginosis. *Front. Cell. Infect. Microbiol.*, 11, 631972. doi:10.3389/fcimb.2021.631972
- [REF:levsagie2022] Lev-Sagie A, De Seta F, Verstraelen H, Ventolini G, Lonnee-Hoffmann R & Vieira-Baptista P (2022). The vaginal microbiome: II. Vaginal dysbiotic conditions. *J. Low. Genit. Tract Dis.*, 26(1), 79–84. doi:10.1097/LGT.0000000000000644
- [REF:price2020] Price TK, Hilt EE, Thomas-White K, Mueller ER, Wolfe AJ & Brubaker L (2020). The urobiome of continent adult women: a cross-sectional study. *BJOG*, 127(2), 193–201. doi:10.1111/1471-0528.15920
- [REF:wolfe2022] Joyce C, Halverson T, Gonzalez C, Brubaker L & Wolfe AJ (2022). The urobiomes of adult women with various lower urinary tract symptoms status differ: a re-analysis. *Front. Cell. Infect. Microbiol.*, 12, 860408. doi:10.3389/fcimb.2022.860408
- [REF:kenneally2022] Kenneally C, Murphy CP, Sleator RD & Culligan EP (2022). The urinary microbiome and biological therapeutics: novel therapies for urinary tract infections. *Microbiol. Res.*, 259, 127010. doi:10.1016/j.micres.2022.127010
- [REF:thomas-white2020] Thomas-White K, Taege S, Limeira R, Brincat C, Joyce C, Hilt EE, ... & Wolfe AJ (2020). Vaginal estrogen therapy is associated with increased *Lactobacillus* in the urine of postmenopausal women with overactive bladder symptoms. *Am. J. Obstet. Gynecol.*, 223(5), 727.e1–727.e11. doi:10.1016/j.ajog.2020.08.006
- [REF:gudnadottir2022] Gudnadottir U, Debelius JW, Du J, Hugerth LW, Danielsson H, Schuppe-Koistinen I, Fransson E & Brusselaers N (2022). The vaginal microbiome and the risk of preterm birth: a systematic review and network meta-analysis. *Sci. Rep.*, 12(1), 7926. doi:10.1038/s41598-022-12007-9
- [REF:huang2023] Huang C, Gin C, Fettweis J, Foxman B, Gelaye B, MacIntyre DA, ... & Callahan B (2023). Meta-analysis reveals the vaginal microbiome is a better predictor of earlier than later preterm birth. *BMC Biol.*, 21(1), 199. doi:10.1186/s12915-023-01702-2
- [REF:ihmp2019] The Integrative Human Microbiome Project Research Network Consor-

- tium (2019). The Integrative Human Microbiome Project. *Nature*, 569(7758), 641–648. doi:10.1038/s41586-019-1238-8
- [REF:plottel2011] Plottel CS & Blaser MJ (2011). Microbiome and malignancy. *Cell Host Microbe*, 10(4), 324–335. doi:10.1016/j.chom.2011.10.003
 - [REF:razavi2019] Razavi AC, Potts KS, Kelly TN & Bazzano LA (2019). Sex, gut microbiome, and cardiovascular disease risk. *Biol. Sex Differ.*, 10(1), 29. doi:10.1186/s13293-019-0240-z
 - [REF:jasarevic2016] Jašarević E, Morrison KE & Bale TL (2016). Sex differences in the gut microbiome-brain axis across the lifespan. *Philos. Trans. R. Soc. Lond. B Biol. Sci.*, 371(1688), 20150122. doi:10.1098/rstb.2015.0122
 - [REF:wu2024] Wu Z, Huang Y, Zhang R, Zheng C, You F, Wang M, Xiao C & Li X (2024). Sex differences in colorectal cancer: with a focus on sex hormone-gut microbiome axis. *Cell Commun. Signal.*, 22(1), 167. doi:10.1186/s12964-024-01549-2
 - Sadeghpour Heravi F (2024). Host-vaginal microbiota interaction: shaping the vaginal microenvironment. *Curr. Clin. Microbiol. Rep.* doi:10.1007/s40588-024-00227-8

Ch. 9 — The Oral Microbiome

- Samman M et al. (2022). Diet drinks and dental caries among U.S. adults. *Community Dent. Health*. doi:10.1922/CDH_00154Samman07

Ch. 10 — The Mycobiome

- [REF:iliev2012] Iliev ID et al. (2012). Interactions between commensal fungi and the C-type lectin receptor Dectin-1 influence colitis. *Science*, 336(6086), 1314–1317. doi:10.1126/science.1221789
- [REF:zhang2022] Zhang F, Aschenbrenner D, Yoo JY & Zuo T (2022). The gut mycobiome in health, disease, and clinical applications. *Lancet Microbe*, 3(12), e969–e983. doi:10.1016/S2666-5247(22)00203-8
- [REF:findley2013] Findley K, Oh J, Yang J, Conlan S et al. (2013). Topographic diversity of fungal and bacterial communities in human skin. *Nature*, 498(7454), 367–370. doi:10.1038/nature12171
- [REF:iliev2013] Iliev ID & Underhill DM (2013). Striking a balance: fungal commensalism versus pathogenesis. *Curr. Opin. Microbiol.*, 16(3), 366–373. doi:10.1016/j.mib.2013.05.004
- [REF:doron2021] Doron I, Leonardi I, Li XV et al. (2021). Human gut mycobiota tune immunity via CARD9-dependent induction of anti-fungal IgG antibodies. *Cell*, 184(4), 1017–1031.e14. doi:10.1016/j.cell.2021.01.016
- [REF:sokol2017] Sokol H, Leducq V, Aschard H et al. (2017). Fungal microbiota dysbiosis in IBD. *Gut*, 66(6), 1039–1048. doi:10.1136/gutjnl-2015-310746
- [REF:noverr2004] Noverr MC, Noggle RM, Toews GB & Huffnagle GB (2004). Role of antibiotics and fungal microbiota in driving pulmonary allergic responses. *Infect. Immun.*, 72(9), 4996–5003. doi:10.1128/IAI.72.9.4996-5003.2004
- [REF:noverr2005] Noverr MC, Falkowski NR, McDonald RA, McKenzie AN & Huffnagle GB (2005). Development of allergic airway disease in mice following antibiotic therapy and fungal microbiota increase. *Infect. Immun.*, 73(1), 30–38. doi:10.1128/IAI.73.1.30-38.2005
- [REF:jung2023] Jung WH (2023). Alteration in skin mycobiome due to atopic dermatitis and seborrheic dermatitis. *Biophys. Rev.*, 4(1), 011309. doi:10.1063/5.0136543
- [REF:belvoncikova2022] Belvončíková P et al. (2022). The human mycobiome: colonization, composition and the role in health and disease. *J. Fungi*, 8(10), 1046.

doi:10.3390/jof8101046

- [REF:wang2022] Wang Y-R, Zhu T, Kong F-Q et al. (2022). Infant mode of delivery shapes the skin mycobiome of prepubescent children. *Microbiol. Spectr.*, 10(5), e02267-22. doi:10.1128/spectrum.02267-22
- [REF:ward2017] Ward TL, Knights D & Gale CA (2017). Infant fungal communities: current knowledge and research opportunities. *BMC Med.*, 15(1), 30. doi:10.1186/s12916-017-0802-z
- [REF:gutierrez2022] Gutierrez MW, van Tilburg Bernardes E et al. (2022). “Molding” immunity — modulation of mucosal and systemic immunity by the intestinal mycobiome. *Mucosal Immunol.*, 15(4), 573–583. doi:10.1038/s41385-022-00515-w
- [REF:gutierrez2025] Gutierrez MW, van Tilburg Bernardes E, Ren E et al. (2025). Early-life gut mycobiome core species modulate metabolic health in mice. *Nat. Commun.*, 16(1), 1467. doi:10.1038/s41467-025-56743-8
- [REF:he2025] He S, Sun Y, Yu J et al. (2025). Dysbiotic gut fungi exacerbate *Klebsiella pneumoniae* lung infection via Dectin-1-mediated alveolar macrophage hyperactivation. *ISME J.*, 19(1). doi:10.1093/ismejo/wraf181
- [REF:hsu2023] Hsu C, Ghannoum M, Cominelli F & Di Martino L (2023). Mycobiome and inflammatory bowel disease: role in disease pathogenesis. *Inflamm. Bowel Dis.*, 29(3), 470–479. doi:10.1093/ibd/izac156
- D’Andreano S et al. (2021). Rapid identification of fungi with long amplicon nanopore sequencing. *Biol Methods Protoc.* doi:10.1093/biomethods/bpaa026
- Money NP (2021). Hyphal and mycelial consciousness: the concept of the fungal mind. *Fungal Biol.* doi:10.1016/j.funbio.2021.02.001
- Lovett B et al. (2020). Behavioral betrayal: How select fungal parasites enlist living insects. *PLoS Pathog.* doi:10.1371/journal.ppat.1008598

Ch. 11 — The Brain Microbiome

- Heller J (2024). The brain might also have a microbiome — what you need to know.
- Link CD (2021). Is there a brain microbiome? *Neurosci. Insights.* doi:10.1177/26331055211018709
- Yong SJ (2024). What If Something Else Lives in Your Brain? The Science and Secrets of Brain Microbiome.
- Hu Z et al. (2023). The remarkable complexity of the brain microbiome in health and disease. *bioRxiv.* doi:10.1101/2023.02.06.527297
- Moné Y et al. (2023). Evidence for a bacterial component in the etiology of Alzheimer’s disease. *Front. Cell. Infect. Microbiol.* doi:10.3389/fcimb.2023.1123228

Ch. 13 — The Gut-Brain Axis

- Cox TO et al. (2026). Intestinal interoceptive dysfunction drives age-associated cognitive decline. *Nature.* doi:10.1038/s41586-026-10191-6
- Cheng YT & Mazmanian SK (2026). Gut microbes affect cognition during ageing. doi:10.1038/d41586-026-00492-1
- Cryan JF & Dinan TG (2012). Mind-altering microorganisms: the gut microbiota on brain and behaviour. *Nat. Rev. Neurosci.* doi:10.1038/nrn3346
- Cryan JF et al. (2020). The gut microbiome in neurological disorders. *Lancet Neurol.* doi:10.1016/S1474-4422(19)30356-4

- Torres-Fuentes C et al. (2017). The microbiota-gut-brain axis in obesity. *Lancet Gastroenterol. Hepatol.* doi:10.1016/S2468-1253(17)30147-4
- Fekete M et al. (2024). Gut-brain axis modulation on cognitive health: prebiotics, probiotics, and symbiotics. *Nutrients.* doi:10.3390/nu16060789
- Meyer K et al. (2022). Gut Microbiota and cognitive function in midlife. *JAMA Netw. Open.* doi:10.1001/jamanetworkopen.2021.43941
- DOE/PNNL (2020). Scientists explore links between genetics, gut microbiome and memory. *Science Daily.*
- Willyard C (2021). How gut microbes could drive brain disorders. *Nature.* doi:10.1038/d41586-021-00260-3
- Mehta RS et al. (2022). Midlife antibiotic use and subsequent cognitive function in women. *PLoS One.* doi:10.1371/journal.pone.0264649

Ch. 14 — Behavioural Modulation

- Hakimi MA et al. (2017). Toxoplasma effectors targeting host signaling and transcription. *Clin. Microbiol. Rev.* doi:10.1128/CMR.00005-17
- Johnson HJ & Koshy AA (2020). Latent toxoplasmosis effects on rodents and humans. *MBio.* doi:10.1128/mBio.02164-19
- de Haan L et al. (2021). Toxoplasma gondii seropositivity and cognitive function: systematic review and meta-analysis. *JAMA Psychiatry.* doi:10.1001/jamapsychiatry.2021.1590
- Stock AK et al. (2017). Humans with latent toxoplasmosis display altered reward modulation. *Sci. Rep.* doi:10.1038/s41598-017-10926-6
- Ortiz-Guerrero G et al. (2020). Cognitive impairment and neurodegeneration by Toxoplasma gondii. *Brain Sci.* doi:10.3390/brainsci10060369
- Rougier S et al. (2017). Lifelong Persistence of Toxoplasma Cysts: A Questionable Dogma? *Trends Parasitol.* doi:10.1016/j.pt.2016.10.007
- Sutterland AL et al. (2015). Toxoplasma gondii in schizophrenia, bipolar disorder, and addiction: meta-analysis. *Acta Psychiatr. Scand.* doi:10.1111/acps.12423

Ch. 15 — Metabolism, Hormones, and the Endocrine Microbiome

- Yue F et al. (2022). Constant light exposure alters gut microbiota and aggravates PCOS-like traits. *Obesity.* doi:10.1002/oby.23380
- Sarmiento J et al. (2022). Nocturnal Light Pollution Induces Weight Gain and Reshapes Colonic Microbiota. *Int. J. Mol. Sci.* doi:10.3390/ijms23031673

Ch. 16–17 — Bacteriophages

- Carroll-Portillo A & Lin HC (2019). Bacteriophage and the innate immune system. *Microorganisms.* doi:10.3390/microorganisms7120625
- Dutilh BE et al. (2014). Highly abundant bacteriophage in unknown sequences of human faecal metagenomes. *Nat. Commun.* doi:10.1038/ncomms5498
- Bondy-Denomy J & Davidson AR (2014). When a virus is not a parasite: beneficial effects of prophages. *J. Microbiol.* doi:10.1007/s12275-014-4083-3
- Atanasova NS et al. (2015). Comparison of lipid-containing bacterial and archaeal viruses. *Adv. Virus Res.* doi:10.1016/bs.aivir.2014.11.005

- Zheludev IN et al. (2024). Viroid-like colonists of human microbiomes [“Obelisks”]. *bioRxiv*. doi:10.1101/2024.01.20.576352
- Sidik S (2024). ‘Wildly weird’ RNA bits discovered infesting the microbes in our guts. *Nature*. doi:10.1038/d41586-024-00266-7
- Thompson J (n.d.). Weird ‘obelisks’ found in human gut may be virus-like entities. *Sci. Am.*
- Raoult D & Forterre P (2008). Redefining viruses: lessons from Mimivirus. *Nat. Rev. Microbiol.* doi:10.1038/nrmicro1858

Ch. 18 — Phage Therapy

- Romero-Calle D et al. (2019). Bacteriophages as alternatives to antibiotics in clinical care. *Antibiotics*. doi:10.3390/antibiotics8030138
- Kushwaha SO et al. (2024). Bacteriophages as potential substitute for antibiotics: Comprehensive review. *Cell Biochem. Funct.* doi:10.1002/cbf.4022
- Petrenko VA (2024). Phage display’s prospects for early diagnosis of prostate cancer. *Viruses*. doi:10.3390/v16020277

Ch. 19 — Antibiotics: Collateral Damage

- Baldanzi G et al. (2026). Antibiotic use and gut microbiome composition: 14,979 individuals. *Nat. Med.* doi:10.1038/s41591-026-04284-y
- Muñoz KA et al. (2024). A Gram-negative-selective antibiotic that spares the gut microbiome. *Nature*. doi:10.1038/s41586-024-07502-0
- Althubiani AA et al. (2024). Antibiotic-induced neuropsychiatric toxicity. *Drugs Context*. doi:10.7573/dic.2024-3-3
- Livanos AE et al. (2016). Antibiotic-mediated gut microbiome perturbation accelerates T1D in mice. *Nat Microbiol.* doi:10.1038/nmicrobiol.2016.140
- Patrick DM et al. (2020). Decreasing antibiotic use, gut microbiota, and asthma incidence in children. *Lancet Respir. Med.* doi:10.1016/S2213-2600(20)30052-7
- Llor C et al. (2011). Impact of rapid antigen testing in acute pharyngitis on antibiotic prescription. *Br. J. Gen. Pract.* doi:10.3399/bjgp11X572436
- von Dach E et al. (2020). CRP-guided antibiotic duration in Gram-negative bacteremia. *JAMA*. doi:10.1001/jama.2020.6348
- Rhee C et al. (2020). Antibiotic-resistant pathogens in culture-proven sepsis. *JAMA Netw Open*. doi:10.1001/jamanetworkopen.2020.2899
- Ternák G et al. (2022). Antibiotic consumption patterns and diabetes prevalence in Europe. *Front. Endocrinol.* doi:10.3389/fendo.2022.870465
- Shuai M et al. (2022). Human Gut Antibiotic Resistome and Progression of Diabetes. *Adv. Sci.* doi:10.1002/advs.202104965
- Jabbar A & Sajjad-Ur-Rehman (2013). Antibiotic residues in meat, milk and eggs. *J Microb Biotech Food Sci.*
- Chowdhury S et al. (2015). Antibiotic residues in milk and eggs in Chittagong, Bangladesh. *Vet World*. doi:10.14202/vetworld.2015.467-471
- Boursi B et al. (2015). Recurrent antibiotic exposure may promote cancer formation. *Eur. J. Cancer*.
- Park SJ et al. (2021). Antibiotics use and diabetes incidence in Korean cohort. *Sci. Rep.* doi:10.1038/s41598-021-01125-5
- Nguyen K et al. (2013). Antibiotic use and resistance in Viet Nam. *BMC Public Health*.

Ch. 20 — Diet: Feeding the Ecosystem

Primary references cited in Chapter 20 draft

- Asnicar F et al. (2021). Microbiome connections with host metabolism and habitual diet from 1,098 deeply phenotyped individuals. *Nat. Med.* doi:10.1038/s41591-020-01183-8
- Bennet SMP et al. (2018). Multivariate modelling of faecal bacterial profiles and food intake in IBS. *Gut.* doi:10.1136/gutjnl-2016-313128
- Berry SE et al. (2020). Human postprandial responses to food and potential for precision nutrition. *Nat. Med.* doi:10.1038/s41591-020-0934-0
- Bielik V, Kolisek M (2021). Bioaccessibility and bioavailability of minerals in relation to a healthy gut microbiome. *Int. J. Mol. Sci.* doi:10.3390/ijms22136803
- Biesiekierski JR et al. (2013). No effects of gluten in patients with self-reported non-celiac gluten sensitivity after dietary reduction of FODMAPs. *Gastroenterology.* doi:10.1053/j.gastro.2013.04.051
- Chassaing B et al. (2015). Dietary emulsifiers impact the mouse gut microbiota promoting colitis and metabolic syndrome. *Nature.* doi:10.1038/nature14232
- Chassaing B et al. (2022). Randomized controlled-feeding study of dietary emulsifier CMC. *Gastroenterology.* doi:10.1053/j.gastro.2021.11.006
- Cortés-Martín A et al. (2020). Polyphenols and gut microbiota metabolites. *Mol. Nutr. Food Res.* doi:10.1002/mnfr.201900952
- David LA et al. (2014). Diet rapidly and reproducibly alters the human gut microbiome. *Nature.* doi:10.1038/nature12820
- Davis DR, Epp MD, Riordan HD (2004). Changes in USDA food composition data for 43 garden crops, 1950 to 1999. *J. Am. Coll. Nutr.* doi:10.1080/07315724.2004.10719409
- Deehan EC et al. (2024). Effects of dietary fibre on metabolic health and obesity. *Nat. Rev. Gastroenterol. Hepatol.* doi:10.1038/s41575-023-00891-z
- Desai MS et al. (2016). A dietary fiber-deprived gut microbiota degrades the colonic mucus barrier. *Cell.* doi:10.1016/j.cell.2016.10.043
- Devkota S et al. (2012). Dietary-fat-induced taurocholic acid promotes pathobiont expansion and colitis. *Nature.* doi:10.1038/nature11225
- Furusawa Y et al. (2013). Commensal microbe-derived butyrate induces Treg differentiation. *Nature.* doi:10.1038/nature12721
- Ghosh TS et al. (2020). Mediterranean diet intervention alters the gut microbiome in older people (NU-AGE). *Gut.* doi:10.1136/gutjnl-2019-319654
- Halmos EP et al. (2014). Diets that differ in FODMAP content alter the colonic luminal microenvironment. *Gut.* doi:10.1136/gutjnl-2014-307264
- Koeth RA et al. (2013). Intestinal microbiota metabolism of L-carnitine promotes atherosclerosis. *Nat. Med.* doi:10.1038/nm.3145
- Koh A et al. (2016). From dietary fiber to host physiology: SCFAs as key bacterial metabolites. *Cell.* doi:10.1016/j.cell.2016.05.041
- Kolodziejczyk AA et al. (2019). Diet-microbiota interactions and personalized nutrition. *Nat. Rev. Microbiol.* doi:10.1038/s41579-019-0256-8
- Lebowitz B et al. (2017). Long-term gluten consumption and coronary heart disease risk. *BMJ.* doi:10.1136/bmj.j1892
- Lönnerdal B (2017). Excess iron intake as a factor in growth, infections, and development of

- infants and young children. *Am. J. Clin. Nutr.* doi:10.3945/ajcn.117.156042
- Mao Q et al. (2018). Ramazzini Institute 13-week pilot study on glyphosate and Roundup in Sprague-Dawley rats: effects on the microbiome. *Environ. Health.* doi:10.1186/s12940-018-0394-x
 - Marco ML et al. (2017). Health benefits of fermented foods: microbiota and beyond. *Curr. Opin. Biotechnol.* doi:10.1016/j.copbio.2016.11.010
 - McDonald D et al. (2018). American Gut: an open platform for citizen science microbiome research. *mSystems.* doi:10.1128/mSystems.00031-18
 - Meslier V et al. (2020). Mediterranean diet intervention in overweight/obese subjects. *Gut.* doi:10.1136/gutjnl-2019-320438
 - Mesnage R et al. (2021). Shotgun metagenomics and metabolomics of glyphosate or Roundup MON 52276 on gut microbiota of Sprague-Dawley rats. *Environ. Health Perspect.* doi:10.1289/EHP6990
 - Mesnage R et al. (2022). Impacts of dietary exposure to pesticides on faecal microbiome metabolism in adult twins. *Environ. Health.* doi:10.1186/s12940-022-00860-0
 - Motta EVS, Raymann K, Moran NA (2018). Glyphosate perturbs the gut microbiota of honey bees. *PNAS.* doi:10.1073/pnas.1803880115
 - Paganini D et al. (2017). Prebiotic GOS mitigate adverse effects of iron fortification on the gut microbiome: RCT in Kenyan infants. *Gut.* doi:10.1136/gutjnl-2017-314418
 - Paganini D et al. (2018). Iron-containing micronutrient powders modify the effect of oral antibiotics and increase post-antibiotic diarrhoea risk. *Gut.* doi:10.1136/gutjnl-2018-317399
 - Palomar-Cros A et al. (2023). Dietary circadian rhythms and cardiovascular disease risk in NutriNet-Santé. *Nat. Commun.* doi:10.1038/s41467-023-43444-3
 - Ranaivo H et al. (2022). Increasing the diversity of dietary fibers in daily-consumed bread modifies gut microbiota and metabolic profile. *Gut Microbes.* doi:10.1080/19490976.2022.2044722
 - Reynolds A et al. (2019). Carbohydrate quality and human health: systematic reviews and meta-analyses. *Lancet.* doi:10.1016/S0140-6736(18)31809-9
 - Schnorr SL et al. (2014). Gut microbiome of the Hadza hunter-gatherers. *Nat. Commun.* doi:10.1038/ncomms4654
 - Smith PM et al. (2013). SCFAs regulate colonic Treg homeostasis. *Science.* doi:10.1126/science.1241165
 - Smits SA et al. (2017). Seasonal cycling in the gut microbiome of the Hadza. *Science.* doi:10.1126/science.aan4834
 - Sonnenburg ED, Sonnenburg JL (2014). Starving our microbial self: consequences of MAC-deficient diet. *Cell Metab.* doi:10.1016/j.cmet.2014.07.003
 - Sonnenburg ED et al. (2016). Diet-induced extinctions in the gut microbiota compound over generations. *Nature.* doi:10.1038/nature16504
 - Staudacher HM, Whelan K (2017). The low FODMAP diet: mechanisms and efficacy in IBS. *Gut.* doi:10.1136/gutjnl-2017-313750
 - Suez J et al. (2014). Artificial sweeteners induce glucose intolerance by altering gut microbiota. *Nature.* doi:10.1038/nature13793
 - Suez J et al. (2022). Personalized microbiome-driven effects of non-nutritive sweeteners on human glucose tolerance. *Cell.* doi:10.1016/j.cell.2022.07.016
 - Taylor BC et al. (2020). Consumption of fermented foods is associated with systematic differences in gut microbiome and metabolome. *mSystems.* doi:10.1128/mSystems.00901-19
 - Thaïss CA et al. (2014). Transkingdom control of microbiota diurnal oscillations promotes metabolic homeostasis. *Cell.* doi:10.1016/j.cell.2014.09.048
 - Thaïss CA et al. (2016). Microbiota diurnal rhythmicity programs host transcriptome oscillations. *Cell.* doi:10.1016/j.cell.2016.11.003

- Tomás-Barberán FA et al. (2017). Urolithins and metabotypes. *Mol. Nutr. Food Res.* doi:10.1002/mnfr.201500901
- Turróni S et al. (2016). Fecal metabolome of the Hadza hunter-gatherers. *Sci. Rep.* doi:10.1038/srep32826
- Walsh L, Hill C, Ross RP (2023). Impact of glyphosate (Roundup) on the composition and functionality of the gut microbiome. *Gut Microbes.* doi:10.1080/19490976.2023.2263935
- Wang Z et al. (2011). Gut flora metabolism of phosphatidylcholine promotes cardiovascular disease. *Nature.* doi:10.1038/nature09922
- Wastyk HC et al. (2021). Gut-microbiota-targeted diets modulate human immune status. *Cell.* doi:10.1016/j.cell.2021.06.019
- Wu GD et al. (2011). Linking long-term dietary patterns with gut microbial enterotypes. *Science.* doi:10.1126/science.1208344
- Zeb F et al. (2020). Time-restricted feeding, metabolic risk, and circadian rhythm. *Br. J. Nutr.* doi:10.1017/S0007114519003428
- Zeevi D et al. (2015). Personalized nutrition by prediction of glycemic responses. *Cell.* doi:10.1016/j.cell.2015.11.001
- Zinöcker MK, Lindseth IA (2018). The Western diet-microbiome-host interaction and metabolic disease. *Nutrients.* doi:10.3390/nu10030365

Additional curated references (not yet cited in draft — reserve for possible inclusion)

- Li H et al. (2024). Resistant starch intake and weight loss by reshaping gut microbiota. *Nat Metab.* doi:10.1038/s42255-024-00988-y
- Lane MM et al. (2024). Ultra-processed food exposure and adverse health outcomes: umbrella review. *BMJ.* doi:10.1136/bmj-2023-077310
- Czarnecka K et al. (2021). Aspartame — True or False? *Nutrients.* doi:10.3390/nu13061957
- Mohr AE et al. (2024). Gut microbiome remodeling: protein pacing with intermittent fasting. *Nat. Commun.* doi:10.1038/s41467-024-48355-5
- Corbin KD et al. (2023). Host-diet-gut microbiome interactions and energy balance. *Nat. Commun.* doi:10.1038/s41467-023-38778-x
- Choi J et al. (2025). Yogurt promotes microbial diversity. *Front. Nutr.* doi:10.3389/fnut.2025.1609102
- Collins J et al. (2018). Dietary trehalose enhances virulence of epidemic *C. difficile*. *Nature.* doi:10.1038/nature25178
- Mesnage R & Antoniou MN (2017). Facts and fallacies in the debate on glyphosate toxicity. *Front. Public Health.* doi:10.3389/fpubh.2017.00316

Ch. 21 — Environment, Lifestyle, and the Modern World

- Huang D et al. (2022). Polystyrene microplastic exposure induces insulin resistance via dysbacteriosis. *Sci. Total Environ.* doi:10.1016/j.scitotenv.2022.155937
- Li D et al. (2020). Microplastic release from polypropylene feeding bottles. *Nature Food.* doi:10.1038/s43016-020-00171-y
- Zrimec J et al. (2021). Plastic-Degrading Potential across the Global Microbiome. *MBio.* doi:10.1128/mBio.02155-21
- Lu J et al. (2018). Triclosan induces multiple antibiotic resistance through genetic mutation. *Environ. Int.* doi:10.1016/j.envint.2018.06.004
- Short FL et al. (2021). Benzalkonium chloride antagonises aminoglycoside antibiotics. *EBio-Medicine.* doi:10.1016/j.ebiom.2021.103653

- Singh R et al. (2019). Lifestyle-Induced Microbial Gradients: An Indian Perspective. *Front. Microbiol.* doi:10.3389/fmicb.2019.02874
- Oduaran OH et al. (2020). Gut Microbiome of Rural and Urban South African Cohort. *bioRxiv*.
- Tomiyama AJ (2019). Stress and Obesity. *Annu. Rev. Psychol.* doi:10.1146/annurev-psych-010418-102936

Ch. 22 — Drugs Beyond Antibiotics

Primary references cited in Chapter 22 draft

Foundational cross-class screens

- [REF:maier2018] Maier L, Pruteanu M, Kuhn M, et al. (2018). Extensive impact of non-antibiotic drugs on human gut bacteria. *Nature*, 555(7698), 623–628. doi:10.1038/nature25979 (PMID: 29555994)
- [REF:vichvila2020] Vich Vila A, Collij V, Sanna S, et al. (2020). Impact of commonly used drugs on the composition and metabolic function of the gut microbiota. *Nat. Commun.*, 11(1), 362. doi:10.1038/s41467-019-14177-z (PMID: 31953381)
- [REF:garcia2024] Garcia-Santamarina S, et al. (2024). Emergence of community behaviors in the gut microbiota upon drug treatment. *Cell*, 187(22), 6346–6357.e20. doi:10.1016/j.cell.2024.08.037

PPIs

- [REF:imhann2016] Imhann F, Bonder MJ, Vich Vila A, et al. (2016). Proton pump inhibitors affect the gut microbiome. *Gut*, 65(5), 740–748. doi:10.1136/gutjnl-2015-310376 (PMID: 26657899)
- [REF:shin2002ppi] Shin JM, Sachs G. (2002). Restoration of acid secretion following treatment with proton pump inhibitors. *Gastroenterology*, 123(5), 1588–1597. doi:10.1053/gast.2002.36593 (PMID: 12404233)
- [REF:zhang2025ppi] Zhang M, et al. (2025). Proton pump inhibitor increases intestinal epithelial paracellular permeability via the p38-MAPK/NF-κB signaling pathway. *Mol. Biol. Rep.*, 53(1), 170. doi:10.1007/s11033-025-11336-y
- [REF:xu2025ppi] Xu J, et al. (2025). Unraveling gut microbiome interferences in cancer immunotherapy: a meta-analysis of diverse drug effects. *BMC Cancer*, 25, 1776. doi:10.1186/s12885-025-15094-9
- [REF:sharma2025ppi] Sharma S, Rawal P, Kalra S. (2025). Gut Guardianship: Recommendations for Rational Use of Proton Pump Inhibitors in Chronic Kidney Disease. *J. Pak. Med. Assoc.*, 75(11), 1822–1824. doi:10.47391/JPKMA.25-94

Metformin

- [REF:forslund2015] Forslund K, Hildebrand F, Nielsen T, et al. (2015). Disentangling type 2 diabetes and metformin treatment signatures in the human gut microbiota. *Nature*, 528(7581), 262–266. doi:10.1038/nature15766 (PMID: 26633628)
- [REF:su2025metformin] Su W, et al. (2025). The Microbial Regulation Spectrum of Metformin in Patients With Type 2 Diabetes: An Individual-Based Meta-Analysis of 1431 Participants. *J. Clin. Endocrinol. Metab.*, 110(8), 2383–2403. doi:10.1210/clinem/dgaf238
- [REF:attaye2024] Attaye I, et al. (2024). Oral Akkermansia muciniphila L2-7 augments glycemic control in type 2 diabetes. *iScience*, 27(8), 110455. doi:10.1016/j.isci.2024.110455

- [REF:xie2024metformin] Xie X, et al. (2024). Metformin reprograms tryptophan metabolism via gut microbiome-derived bile acid metabolites to ameliorate depression-like behaviors in mice. *Brain Behav. Immun.*, 123, 442–455. doi:10.1016/j.bbi.2024.09.014
- [REF:rosell2024] Rosell-Díaz M, et al. (2024). Metformin-induced changes in the gut microbiome and plasma metabolome are associated with cognition in men. *Metabolism*, 157, 155941. doi:10.1016/j.metabol.2024.155941

NSAIDs

- [REF:varela2025] Varela-Mendoza AA, et al. (2025). Kombucha Prevents Indomethacin-Induced Enteric Damage in Wistar Rat by Enhancing Epithelial Gut Barrier and Modulating Gut Microbiota. *Food Sci. Nutr.*, 13(8), e70804. doi:10.1002/fsn3.70804
- [REF:tuerxuntayi2025] Tuerxuntayi A, et al. (2025). Paeoniflorin attenuates ASA-induced enteropathy in simulated high-altitude hypoxia in rats by regulating intestinal inflammation, intestinal barrier, and intestinal flora. *Toxicol. Appl. Pharmacol.*, 506, 117641. doi:10.1016/j.taap.2025.117641

Statins

- [REF:oesterle2017] Oesterle A, Laufs U, Liao JK. (2017). Pleiotropic Effects of Statins on the Cardiovascular System. *Circ. Res.*, 120(3), 573–585. doi:10.1161/CIRCRESAHA.116.308537 (PMID: 28154098)
- [REF:cochrane2019eze] Defined SR, et al. (2019). Ezetimibe for the prevention of cardiovascular disease and all-cause mortality events. *Cochrane Database Syst. Rev.*, 2019(11), CD012502. doi:10.1002/14651858.CD012502.pub2
- [REF:khan2023pcsk9] Khan SU, et al. (2023). PCSK-9 Inhibitors and Cardiovascular Outcomes: A Systematic Review With Meta-Analysis. *Cardiovasc. Ther.*, 2023, 4653180. doi:10.1155/2023/4653180
- [REF:vieirasilva2020] Vieira-Silva S, Falony G, Belda E, et al. (2020). Statin therapy is associated with lower prevalence of gut microbiota dysbiosis. *Nature*, 581(7808), 310–315. doi:10.1038/s41586-020-2269-x (PMID: 32433607)
- [REF:verhaar2022] Verhaar BJH, Collard D, Prodan A, et al. (2022). Heterogeneity in statin responses explained by variation in the human gut microbiome. *Med*, 3(6), 388–405.e6. doi:10.1016/j.medj.2022.05.002
- [REF:lind2024] Lind L, et al. (2024). Is LDL cholesterol associated with long-term mortality among primary prevention adults? A retrospective cohort study from a large healthcare system. *BMJ Open*, 14(3), e080151. doi:10.1136/bmjopen-2023-080151
- [REF:liu2021nhanes] Liu X, et al. (2021). Association between low density lipoprotein cholesterol and all-cause mortality: results from the NHANES 1999–2014. *Sci. Rep.*, 11(1), 22111. doi:10.1038/s41598-021-01738-w
- [REF:liu2025statin] Liu YH, et al. (2025). Gut microbiota profiles by LDL-C target achievement in statin-treated patients: A cross-sectional study. *Nutr. Metab. Cardiovasc. Dis.*, 36(3), 104465. doi:10.1016/j.numecd.2025.104465

Antipsychotics and antidepressants

- [REF:kamath2024] Kamath S, et al. (2024). The atypical antipsychotics lurasidone and olanzapine exert contrasting effects on the gut microbiome and metabolic function of rats. *Br. J. Pharmacol.*, 181(22), 4531–4545. doi:10.1111/bph.16507
- [REF:qian2023olanzapine] Qian L, et al. (2023). Longitudinal Gut Microbiota Dysbiosis Underlies Olanzapine-Induced Weight Gain. *Microbiol. Spectr.*, 11(4), e0005823.

doi:10.1128/spectrum.00058-23

- [REF:zeng2024olanzapine] Zeng C, et al. (2024). B-GOS alleviates olanzapine-induced lipid disturbances in mice by enriching Akkermansia and upregulation of PGRMC1-Wnt signaling. *Food Chem. Toxicol.*, 185, 114490. doi:10.1016/j.fct.2024.114490
- [REF:bertossi2024] Bertossi F. (2024). A Possible Role of Akkermansia muciniphila in the Treatment of Olanzapine-Induced Weight Gain. *Cureus*, 16(3), e55733. doi:10.7759/cureus.55733
- [REF:dias2024] Dias MF, et al. (2024). Effects of antipsychotics on the gastrointestinal microbiota: A systematic review. *Psychiatry Res.*, 336, 115914. doi:10.1016/j.psychres.2024.115914

Laxatives

- [REF:tropini2018] Tropini C, Moss EL, Merrill BD, et al. (2018). Transient osmotic perturbation causes long-term alteration to the gut microbiota. *Cell*, 173(7), 1742–1754.e17. doi:10.1016/j.cell.2018.05.008 (PMID: 29906449)

Hormonal contraceptives and HRT

- [REF:hua2022] Hua X, et al. (2022). Longitudinal analysis of the impact of oral contraceptive use on the gut microbiome. *J. Med. Microbiol.*, 71(4). doi:10.1099/jmm.0.001512
- [REF:brito2025] Brito J, et al. (2025). Hormonal birth control is associated with altered gut microbiota β -diversity in physically active females across the menstrual cycle: a pilot trial. *J. Appl. Physiol.*, 138(3), 739–745. doi:10.1152/japplphysiol.00008.2025
- [REF:clapp2025] Clapp Organski A, et al. (2025). Oral Contraceptives Induce Time- and Intestinal Segment-Dependent Shifts in the Gut Microbiota. *Nutrients*, 17(16), 2591. doi:10.3390/nu17162591

Iron, vitamin D, fish oil, and herbal supplements

- [REF:baldi2024] Baldi A, et al. (2024). Effects of iron supplements and iron-containing micronutrient powders on the gut microbiome in Bangladeshi infants: a randomized controlled trial. *Nat. Commun.*, 15(1), 8640. doi:10.1038/s41467-024-53013-x
- [REF:tian2025iron] Tian C, et al. (2025). Intramuscular iron supplementation enhances intestinal barrier function in weaned piglets challenged with enterotoxigenic *E. coli*. *Front. Cell. Infect. Microbiol.*, 15, 1553639. doi:10.3389/fcimb.2025.1553639
- [REF:feng2025vitd] Feng X, et al. (2025). Vitamin D Deficiency Exacerbates d-Galactose-Induced Intestinal Barrier Dysfunction in a Murine Model of Intestinal Aging. *J. Agric. Food Chem.*, 73(39), 24725–24740. doi:10.1021/acs.jafc.5c07085
- [REF:wu2025vitd] Wu CW, et al. (2025). Vitamin D3 Improves Hypothalamic-Pituitary-Adrenal Axis Function, Immunological Responses, and Gut Dysbiosis in Sleep Desynchrony. *Brain Behav.*, 15(11), e71084. doi:10.1002/brb3.71084
- [REF:cao2025omega3] Cao B, et al. (2025). Interaction between dietary omega-3 polyunsaturated fatty acids, obesity and gut microbiota in preclinical models: A systematic review of randomized controlled trials. *Diabetes Obes. Metab.*, 27(9), 4643–4661. doi:10.1111/dom.16535
- [REF:roussel2025fishoil] Roussel C, et al. (2025). Human Gut Microbes Produce EPA- and DHA-Derived Oxylipins, but not N-Acyl-Ethanolamines, From Fish Oil. *FASEB J.*, 39(12), e70713. doi:10.1096/fj.202500752RR
- [REF:guo2023berberine] Guo HH, et al. (2023). Berberine is a potential alternative for metformin with good regulatory effect on lipids in treating metabolic diseases. *Biomed. Pharmacother.*, 163, 114754. doi:10.1016/j.biopha.2023.114754

- [REF:kong2020berberine] Kong WJ, et al. (2020). Berberine in the treatment of metabolism-related chronic diseases: A drug cloud (dCloud) effect to target multifactorial disorders. *Pharmacol. Ther.*, 209, 107496. doi:10.1016/j.pharmthera.2020.107496

Pharmacomicrobiomics — drugs metabolised by microbes

- [REF:zimmermann2019] Zimmermann M, Zimmermann-Kogadeeva M, Wegmann R, Goodman AL. (2019). Mapping human microbiome drug metabolism by gut bacteria and their genes. *Nature*, 570(7762), 462–467. doi:10.1038/s41586-019-1291-3 (PMID: 31158845)
- [REF:haiser2013] Haiser HJ, Gootenberg DB, Chatman K, Sirasani G, Balskus EP, Turnbaugh PJ. (2013). Predicting and manipulating cardiac drug inactivation by the human gut bacterium *Eggerthella lenta*. *Science*, 341(6143), 295–298. doi:10.1126/science.1235872 (PMID: 23869020)
- [REF:mainirekdal2019] Maini Rekdal V, Bess EN, Bisanz JE, Turnbaugh PJ, Balskus EP. (2019). Discovery and inhibition of an interspecies gut bacterial pathway for Levodopa metabolism. *Science*, 364(6445), eaau6323. doi:10.1126/science.aau6323 (PMID: 31196984)

Additional reserve references (not cited in current draft — kept for possible future inclusion)

- Hall RJ et al. (2024). Non-antibiotic pharmaceuticals are toxic against *E. coli*. *NPJ Antimicrob. Resist.* doi:10.1038/s44259-024-00028-5
- Chen H et al. (2025). Non-antibiotic medications and AMR in *E. coli*. *NPJ Antimicrob. Resist.* doi:10.1038/s44259-025-00144-w
- Imhann F et al. (2017). Proton pump inhibitors and other medications on gut microbiota. *Gut Microbes*. doi:10.1080/19490976.2017.1284732
- Le Bastard Q et al. (2021). Impact of non-antibiotic drugs on intestinal microbiome. *Expert Rev. Mol. Diagn.* doi:10.1080/14737159.2021.1952075
- Pyo JH et al. (2021). Proton pump inhibitors and fatty liver disease risk. *J. Gastroenterol. Hepatol.* doi:10.1111/jgh.15236
- Wu H et al. (2017). Metformin alters the gut microbiome in treatment-naïve T2D. *Nat. Med.* doi:10.1038/nm.4345

Ch. 23 — Probiotics: Promise, Hype, and Hard Truths

- Suez J et al. (2018). Post-antibiotic gut mucosal microbiome reconstitution is impaired by probiotics. *Cell*. doi:10.1016/j.cell.2018.08.047
- Butler CC et al. (2020). Probiotic Use and Antibiotic Administration in Care Home Residents. *JAMA*. doi:10.1001/jama.2020.8556
- Li G et al. (2023). Probiotics supplementation and glycaemic control in T2DM: meta-analysis. *J. Transl. Med.* doi:10.1186/s12967-023-04306-0
- Gao K et al. (2023). *Bifidobacterium longum* WHH2270 ameliorates type 2 diabetes in rats. *J. Food Sci.* doi:10.1111/1750-3841.16727
- Forsyth CB et al. (2009). *Lactobacillus* GG treatment ameliorates alcohol-induced liver injury. *Alcohol*. doi:10.1016/j.alcohol.2008.12.009
- Maguire M & Maguire G (2019). Gut dysbiosis, leaky gut, and intestinal epithelial proliferation in neurological disorders. *Rev. Neurosci.* doi:10.1515/revneuro-2018-0024

Ch. 24 — Faecal Microbiota Transplantation

- Udayappan SD et al. (2014). Intestinal microbiota and FMT as treatment for insulin resistance and T2DM. *Clin. Exp. Immunol.* doi:10.1111/cei.12293

Ch. 26 — Inflammatory and Autoimmune Disease

- Tomofuji Y et al. (2021). Gut microbiome of systemic lupus erythematosus in Japanese. *Ann. Rheum. Dis.* doi:10.1136/annrheumdis-2021-220687
- Huang R et al. (2020). Intestinal microbiome in ankylosing spondylitis patients. *J. Med. Microbiol.* doi:10.1099/jmm.0.001107
- Dedrick S et al. (2020). Gut Microbiota and Environmental Factors in T1D Pathogenesis. *Front. Endocrinol.* doi:10.3389/fendo.2020.00078
- Mu Q et al. (2017). Leaky Gut As a Danger Signal for Autoimmune Diseases. *Front. Immunol.* doi:10.3389/fimmu.2017.00598
- Van Dijck C et al. (2021). Antibacterial mouthwash for STIs in MSM on PrEP (PREGo). *Lancet Infect. Dis.* doi:10.1016/S1473-3099(20)30778-7
- Kenyon C et al. (2023). Asymptomatic STI management in Europe. *Lancet Reg. Health Eur.* doi:10.1016/j.lanepe.2023.100743

Ch. 27 — Metabolic Disease and Obesity

Note: This is the primary home for the diabetes/obesity references. The bibliography is rich here — reflecting the author’s research interest — and will provide substantial material for a detailed chapter.

- Qin J et al. (2012). Metagenome-wide association study of gut microbiota in T2D. *Nature.* doi:10.1038/nature11450
- de Vos WM & Nieuwdorp M (2013). A gut prediction. *Nature.* doi:10.1038/nature12251
- Hartstra AV et al. (2014). Role of the Microbiome in Obesity and T2D. *Diabetes Care.* doi:10.2337/dc14-0769
- Tilg H & Moschen AR (2014). Microbiota and diabetes: an evolving relationship. *Gut.* doi:10.1136/gutjnl-2014-306928
- Barlow GM et al. (2015). Role of the Gut Microbiome in Obesity and DM. *Nutr. Clin. Pract.* doi:10.1177/0884533615609896
- Cai L et al. (2015). T2D Biomarkers of Human Gut Microbiota. *PLoS One.* doi:10.1371/journal.pone.0140827
- Sabatino A et al. (2017). Intestinal Microbiota in T2D and CKD. *Curr. Diab. Rep.* doi:10.1007/s11892-017-0841-z
- Tong X et al. (2018). Gut Microbiota during amelioration of T2D with hyperlipidemia by metformin. *MBio.* doi:10.1128/mBio.02392-17
- Liu G et al. (2018). Pumpkin polysaccharide modifies gut microbiota during alleviation of T2D. *Int. J. Biol. Macromol.* doi:10.1016/j.ijbiomac.2018.04.127
- Nie X et al. (2020). Gut microbiome and visceral fat accumulation. *Comput. Struct. Biotechnol. J.* doi:10.1016/j.csbj.2020.09.026
- Liu X et al. (2021). GWAS for gut metagenome in Chinese adults. *Cell Discov.* doi:10.1038/s41421-020-00239-w
- Wang Z et al. (2024). Gut Microbiota and blood metabolites related to fiber and T2D. *Circ. Res.* doi:10.1161/circresaha.123.323634

- Peng D et al. (2023). Dendrobium officinale derivatives in a T2D mouse model. *Food Chem.* doi:10.1016/j.foodchem.2022.133974
- Neri-Rosario D et al. (2023). Dysbiosis signatures and T2D progression: machine learning. *Front. Endocrinol.* doi:10.3389/fendo.2023.1170459
- Gravdal K et al. (2023). Gut microbiota in pre-diabetes and treatment-naïve T2D. *BMC Endocr. Disord.* doi:10.1186/s12902-023-01432-0
- Hu J et al. (2023). Gut microbiota signatures in T1D and T2D. *EClinicalMedicine.* doi:10.1016/j.eclinm.2023.102132
- Zhang L et al. (2023). Intestinal Microbiota in Early and Late Stages of DKD. *Microbiol Spectr.* doi:10.1128/spectrum.00382-23
- Lu X et al. (2023). Gut microbiota in diabetic nephropathy. *Sci. Rep.* doi:10.1038/s41598-023-39444-4
- Chen X et al. (2023). Dendrobium officinale polysaccharide alleviates T2DM. *J. Agric. Food Chem.* doi:10.1021/acs.jafc.3c02429
- Cuadrat RRC et al. (2023). Gut microbiota and vascular stiffness. *Sci. Rep.* doi:10.1038/s41598-023-40178-6
- Antwi J (2023). Precision Nutrition for Obesity and T2D. *Curr. Nutr. Rep.* doi:10.1007/s13668-023-00491-y
- Ofosu FK et al. (2023). Fermented sorghum improves T2D via gut microbiota. *J. Funct. Foods.* doi:10.1016/j.jff.2023.105666
- Han CY et al. (2023). Benaglutide effects on Gut Microbiota in T2DM. *Diab. Metab. Syndr. Obes.* doi:10.2147/DMSO.S418757
- Niu X et al. (2023). Metformin + liraglutide and gut microbiota in T2D. *Int. Microbiol.* doi:10.1007/s10123-023-00380-y
- Zhou H et al. (2023). Diet composition influences cholesterol via gut Microbiota in ApoE^{-/-} mice. *J. Agric. Food Chem.* doi:10.1021/acs.jafc.3c00810
- Pierce DR et al. (2023). Experimental hookworm infection and insulin resistance. *Nat. Commun.* doi:10.1038/s41467-023-40263-4
- Hughes S (2022). Gut microbiome product implicated in heart failure risk.
- Melville NA (2022). Gut microbiome species predict T2D.
- Jie Z et al. (2023). Three-bacteria consortium inhibits atherosclerosis in mice. *iScience.* doi:10.1016/j.isci.2023.106960

Ch. 28 — Cancer

- Boursi B et al. (2015). Recurrent antibiotic exposure may promote cancer formation. *Eur. J. Cancer.*
- Ugai T et al. (2022). Is early-onset cancer an emerging global epidemic? *Nat. Rev. Clin. Oncol.* doi:10.1038/s41571-022-00672-8
- Palomar-Cros A et al. (2023). Artificial sweeteners and cancer risk. *Int. J. Cancer.* doi:10.1002/ijc.34577

Ch. 29 — Neurological and Psychiatric Disease

- Seo DO et al. (2023). ApoE and microbiota-dependent progression of tauopathy. *Science.* doi:10.1126/science.add1236
- Seo DO & Holtzman DM (2020). Gut Microbiota and Alzheimer's pathology. *J. Gerontol.* doi:10.1093/gerona/glz262

- Seo DO & Holtzman DM (2024). Alzheimer's disease-associated microbiome and therapeutic strategies. *Exp. Mol. Med.* doi:10.1038/s12276-023-01146-2
- Seo DO (2019). The microbiome: A target for Alzheimer disease? *Cell Res.* doi:10.1038/s41422-019-0227-7
- Konings B et al. (2023). GI syndromes preceding Parkinson's disease: testing Braak's hypothesis. *Gut.* doi:10.1136/gutjnl-2023-329685
- Zhu F et al. (2020). Gut microbiome and schizophrenia. *Nat. Commun.*

Ch. 31 — Ethical, Social, and Philosophical Questions

- Spencer HG & Zuk M (2016). For Host's Sake: The Pluses of Parasite Preservation. *Trends Ecol. Evol.* doi:10.1016/j.tree.2016.02.021
- Murphy AA et al. (2016). Self-disseminating vaccines for emerging infectious diseases. *Expert Rev. Vaccines.* doi:10.1586/14760584.2016.1106942
- Rosenthal SR et al. (2015). Redefining disease emergence. *One Health.* doi:10.1016/j.onehlt.2015.08.001

Notable Observations

1. **Diabetes/metabolic disease concentration:** ~50 of the 176 references are diabetes-focused. These are concentrated in Ch. 27 (primary home) with relevant spillover into Ch. 22 (metformin), Ch. 19 (antibiotics and T1D), and Ch. 26 (autoimmune T1D). This is rich material for a strong metabolic disease chapter, but as you noted, should not disproportionately dominate the book.
2. **Strong collections for specific chapters:** Toxoplasma/behavioural modulation (Ch. 14), bacteriophages and obelisks (Ch. 16–17), the brain microbiome (Ch. 11), and artificial sweeteners (Ch. 20) all have good clusters of references.
3. **Gaps to fill:** Chapters 6 (skin), 9 (oral), 10 (mycobiome), 24 (FMT), 25 (precision engineering), and 32 (future) have few or no references in this bibliography. These will need additional literature searches during drafting.

Ch. 13 — The Gut-Brain Axis: Microbes and the Mind

- [REF:bravo2011] Bravo JA, Forsythe P, Chew MV, Escaravage E, Savignac HM, Dinan TG, Bienenstock J & Cryan JF (2011). Ingestion of *Lactobacillus* strain regulates emotional behavior and central GABA receptor expression in a mouse via the vagus nerve. *PNAS*, 108(38), 16050–16055. doi:10.1073/pnas.1102999108
- [REF:yano2015] Yano JM, Yu K, Donaldson GP, Shastri GG, Ann P, Ma L, Nagler CR, Ismailov RF, Mazmanian SK & Hsiao EY (2015). Indigenous bacteria from the gut microbiota regulate host serotonin biosynthesis. *Cell*, 161(2), 264–276. doi:10.1016/j.cell.2015.02.047
- [REF:strandwitz2019] Strandwitz P et al. (2019). GABA-modulating bacteria of the human gut microbiota. *Nat. Microbiol.*, 4(3), 396–403. doi:10.1038/s41564-018-0307-3
- [REF:sudo2004] Sudo N, Chida Y, Aiba Y et al. (2004). Postnatal microbial colonization programs the hypothalamic-pituitary-adrenal system for stress response in mice. *J. Physiol.*, 558(Pt 1), 263–275. doi:10.1113/jphysiol.2004.063388

- [REF:bercik2011] Bercik P, Denou E, Collins J et al. (2011). The intestinal microbiota affect central levels of brain-derived neurotrophic factor and behavior in mice. *Gastroenterology*, 141(2), 599–609.e3. doi:10.1053/j.gastro.2011.04.052
- [REF:diazheijt2011] Diaz Heijt R, Wang S, Anuar F et al. (2011). Normal gut microbiota modulates brain development and behavior. *PNAS*, 108(7), 3047–3052. doi:10.1073/pnas.1010529108
- [REF:dinan2013] Dinan TG, Stanton C & Cryan JF (2013). Psychobiotics: a novel class of psychotropic. *Biol. Psychiatry*, 74(10), 720–726. doi:10.1016/j.biopsych.2013.05.001
- [REF:kelly2016] Kelly JR, Borre Y, O'Brien C et al. (2016). Transferring the blues: depression-associated gut microbiota induces neurobehavioural changes in the rat. *J. Psychiatr. Res.*, 82, 109–118. doi:10.1016/j.jpsychires.2016.07.019
- [REF:vallescolomer2019] Valles-Colomer M, Falony G, Darzi Y et al. (2019). The neuroactive potential of the human gut microbiota in quality of life and depression. *Nat. Microbiol.*, 4(4), 623–632. doi:10.1038/s41564-018-0337-x
- [REF:asad2025] Asad A, Kirk M, Zhu S et al. (2025). Effects of prebiotics and probiotics on symptoms of depression and anxiety in clinically diagnosed samples: systematic review and meta-analysis of randomized controlled trials. *Nutr. Rev.*, 83(7), e1504–e1520. doi:10.1093/nutrit/nuae177
- [REF:hsiao2013] Hsiao EY, McBride SW, Hsien S et al. (2013). Microbiota modulate behavioral and physiological abnormalities associated with neurodevelopmental disorders. *Cell*, 155(7), 1451–1463. doi:10.1016/j.cell.2013.11.024
- [REF:sharon2019] Sharon G, Cruz NJ, Kang DW et al. (2019). Human gut microbiota from autism spectrum disorder promote behavioral symptoms in mice. *Cell*, 177(6), 1600–1618.e17. doi:10.1016/j.cell.2019.05.004
- [REF:kang2017] Kang DW, Adams JB, Gregory AC et al. (2017). Microbiota Transfer Therapy alters gut ecosystem and improves gastrointestinal and autism symptoms: an open-label study. *Microbiome*, 5(1), 10. doi:10.1186/s40168-016-0225-7
- [REF:kang2019] Kang DW, Adams JB, Coleman DM et al. (2019). Long-term benefit of Microbiota Transfer Therapy on autism symptoms and gut microbiota. *Sci. Rep.*, 9, 5821. doi:10.1038/s41598-019-42183-0
- [REF:wan2024] Wan YP et al. (2024). Effect of oral faecal microbiota transplantation intervention for children with autism spectrum disorder: a randomised, double-blind, placebo-controlled trial. *Clin. Transl. Med.*, 14, e70006. doi:10.1002/ctm2.70006

Ch. 17 — Phages, Immunity, and the Tripartite Relationship

- [REF:nguyen2017] Nguyen S, Baker K, Padman BS, Patwa R, Dunstan RA, Weston TA, Schlosser K, Bailey B, Lithgow T, Lazarou M, Luque A, Rohwer F, Blumberg RS & Barr JJ (2017). Bacteriophage Transcytosis Provides a Mechanism To Cross Epithelial Cell Layers. *mBio*, 8(6), e01874-17. doi:10.1128/mBio.01874-17
- [REF:barr2013] Barr JJ, Auro R, Furlan M, Whiteson KL, Erb ML, Pogliano J, Stotland A, Wolkowicz R, Cutting AS, Doran KS, Salamon P, Youle M & Rohwer F (2013). Bacteriophage adhering to mucus provide a non-host-derived immunity. *PNAS*, 110(26), 10771–10776. doi:10.1073/pnas.1305923110
- [REF:barr2015] Barr JJ, Auro R, Sam-Soon N, Kassegne S, Peters G, Bonber N, Hatber M, Molineux S, Guardado A, Vega R, Bailey B, Pogliano J, Danthi P & Rohwer F (2015). Subdiffusive motion of bacteriophage in mucosal surfaces increases the frequency of bacterial encounters. *PNAS*, 112(44), 13675–13680. doi:10.1073/pnas.1508355112

- [REF:almeida2019] Almeida GMF, Laanto E, Asber R & Sundberg LR (2019). Bacteriophage Adherence to Mucus Mediates Preventive Protection against Pathogenic Bacteria. *mBio*, 10(6), e01984-19. doi:10.1128/mBio.01984-19
 - [REF:barr2022] Barr JJ et al. (2022). Bacteriophages evolve enhanced persistence to a mucosal surface. *PNAS*, 119(27), e2116197119. doi:10.1073/pnas.2116197119
 - [REF:vanbellegghem2017] Van Bellegghem JD, Clement F, Merabishvili M, Lavigne R & Vaneechoutte M (2017). Pro- and anti-inflammatory responses of peripheral blood mononuclear cells induced by *Staphylococcus aureus* and *Pseudomonas aeruginosa* phages. *Sci. Rep.*, 7, 8004. doi:10.1038/s41598-017-08336-9
 - [REF:vanbellegghem2019] Van Bellegghem JD, Dąbrowska K, Vaneechoutte M, Barr JJ & Bollyky PL (2019). Interactions between Bacteriophage, Bacteria, and the Mammalian Immune System. *Viruses*, 11(1), 10. doi:10.3390/v11010010
 - [REF:gogokhia2019] Gogokhia L, Buber K, Roundy J, Padgett MJ, Hapfelmeier S, Hooper LV & Round JL (2019). Expansion of Bacteriophages Is Linked to Aggravated Intestinal Inflammation and Colitis. *Cell Host Microbe*, 25(2), 285–299. doi:10.1016/j.chom.2019.01.008
 - [REF:gorski2012] Górski A, Międzybrodzki R, Borysowski J et al. (2012). Phage as a Modulator of Immune Responses: Practical Implications for Phage Therapy. *Adv. Virus Res.*, 83, 41–71. doi:10.1016/B978-0-12-394438-2.00002-5
 - [REF:gorski2017] Górski A, Dąbrowska K, Międzybrodzki R, Weber-Dąbrowska B, Łusiak-Szelachowska M, Jończyk-Matysiak E & Borysowski J (2017). Phages and immunomodulation. *Future Microbiol.*, 12(10), 905–914. doi:10.2217/fmb-2017-0049
 - [REF:lamybesnier2024] Lamy-Besnier Q, Theodorou I, Billaud M, Zhang H, Brot L, Culot A, Abriat G, Sokol H, De Paepe M, Petit MA & De Sordi L (2024). The human blood harbors a phageome which differs in Crohn's disease. *bioRxiv* / preprint. doi:10.1101/2024.06.04.597176
 - [REF:phageometransfer2024] (2024). Phageome transfer from gut to circulation and its regulation by human immunity. *bioRxiv* / preprint. doi:10.1101/2024.06.05.597592
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4. **Obelisks** (Zheludev et al. 2024): A fascinating recent discovery — viroid-like RNA elements in the human gut microbiome. This could warrant its own sidebar or sub-section in the phage chapters (Ch. 16–17), as it expands the concept of what biological entities inhabit our microbiome beyond bacteria, fungi, and classical viruses.
 5. **Paternal microbiome** (Argaw-Denboba et al. 2024): This is a Nature paper showing paternal gut microbiome perturbations affect offspring fitness — an important addition to Ch. 3 that expands the maternal-focused narrative of microbial inheritance.
 6. **Harada et al. (2025)**: An ultra-reduced archaeal genome — potentially relevant to Ch. 10 or a general discussion of the minimal requirements for cellular life within the microbiome.