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Prologue: Scrubbing Up

12.46 p.m. GMT on 11 September 2001: I had just finished my first ever appendicectomy in operating room number three at St Mary's Hospital, London, and I was ecstatic. After six long years at medical school, I was finally doing something of actual use. I didn't know that over the next twenty years I would spend many long nights guddling around the dark recesses of people's abdomens trying to find this elusive source of pain. I didn't know that I would become obsessed by the bacteria that live in the gut and their critical importance to the workings of our fragile bodies. And I didn't know that there comes a point in every surgeon's career when they conclude that it would be better for all concerned if there was no need for surgery at all.

You may have lost your appendix to a surgeon like me, and you may have been told that the appendix is an organ without a function – an evolutionary footnote. It's true that you can live perfectly happily without an appendix, but it is most certainly not useless. Every one of our organs, no matter how ridiculous it might look or sound or feel, has evolved and stayed with us for a reason. It's not mere coincidence or bad luck that people who have their appendix taken out as children have an increased risk of developing inflammatory bowel disease, *Clostridium difficile* infections, colorectal cancer and even irritable bowel syndrome (IBS).[1] Just because we haven't been able to define its purpose, we foolishly assume the appendix has none. But the humble appendix contains a multitude of microscopic life forms that were, until recently, completely alien to us. Their discovery means that this enduring mystery of modern science might not remain a mystery for much longer.

On that Tuesday in 2001 I walked out of the operating room and into the coffee area on the fourth floor of the hospital at 1.03 p.m., just as the second plane hit the South Tower of the World Trade

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Center in New York. The whole world stopped, and we silently watched as 2,996 people were murdered. The hospital is on the flight path to Heathrow Airport. Later that night I stood staring out of the ward's window, marvelling at the empty sky, and felt the sense of dread that hung over the city. No one could have imagined how bloody the vengeance for this atrocity would be. The war on terror would cost 900,000 lives and US $8 trillion, displacing thirty-eight million people and their microbes across the globe.[2] The supersized, ready-to-eat, buy-one-get-one-free, instantly delivered digital culture that rode the bow-wave of the global military offensive homogenized the diets and lives of people who did not want it. It was a harmful acceleration of a globalized way of life that had its roots in the Second World War and that has left microbiological chaos in its wake.

A surgeon from the 1800s, asked to perform an appendicectomy in my operating theatre, would be startled by the changes in culture and technology, as well as by the improvements in patient survival. But they would still recognize the fundamentals of the operation, because a great deal of twenty-first-century practice is based on nineteenth-century scientific principles. For several thousand years the leading cause of surgical mortality has been – and still is – infection. However, major breakthroughs and innovations in antisepsis and antibiotics in the nineteenth and twentieth centuries have transformed outcomes from surgical procedures. For my profession, 'scrubbing up' remains a rite of passage and a performance art. It offers time to think, time to mentally rehearse and, occasionally, time to panic. It's one of the most important acts of surgery: bacteria are the enemy, and this choreographed dance rids them from my hands. Most of my clinical practice has been spent in a state of war with micro-organisms and, collectively, the medical profession has prescribed so many antibiotics, so recklessly, that bacteria are now resistant to these precious medicines. The frightening part is that we have no new medicines to replace them with.

Humankind has made extraordinary advances in all aspects of healthcare during this timeframe; for example, your chance of

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surviving cancer has increased by 50 per cent in just fifty years. But it hasn't solved all of our problems, and today society struggles to cope with a startling rise in the diseases and disorders of progress, many of which begin with the letter 'A', such as asthma,[3] allergies,[4] autoimmune diseases[5] and even autism.[6] The pattern of growth in these disorders across populations varies according to factors like our postcode, our wealth and access to increasingly sophisticated detection strategies. However, the steady and undeniable climb in the worldwide prevalence of these conditions can't be explained away by genetic variance or the shifting goalposts of diagnostic definitions. Many of these conditions disproportionately affect the young and those at extremes of age: in 2019, an estimated 38.2 million children under the age of five were overweight or obese and this number is steadily rising, meaning that when they become adults they will carry a disproportionate burden of diabetes and cardiovascular disease, the number-one cause of death worldwide.[7] Generation Z can also expect to have a risk of bowel cancer four times that of someone born in the 1960s.[8] Alzheimer's and dementia prevalence is climbing so fast in our rapidly ageing population that, by 2050, 151 million people worldwide will not be able to recognize their own families.[9]

Alarmingly, the majority of drugs we have to treat these conditions don't work in the majority of people, and the pharma pipelines that are supposed to be creating new treatments for our aching bodies are running perilously dry. As a result, my profession has had to adapt; we haven't only created new procedures – we've developed new surgical specialities. Bariatric surgery for weight loss, for example, didn't exist as a sub-speciality when I qualified; today, more than 250,000 weight-loss surgeries are performed each year in the US alone.

Twenty-first-century living is causing our airways to close, our skin to flake, our joints to swell, our guts to bleed, our arteries to clot and our brains to seize up. The global pandemic of non-infectious diseases is, arguably, a greater threat to humanity than that caused by any communicable disease. The healthcare systems set up to treat these diseases are increasingly unsustainable. If we

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are to meaningfully understand how and why we've reached this crisis – and how to solve it – we urgently need to reappraise our relationship with our microbes.

Throughout my career as a surgeon and clinical scientist I have become increasingly interested in one question: if the microbes that live inside us are so bad for us, why are they there to begin with? Why have so many of them chosen us as their hosts, and why have we offered ourselves so readily to them?

While some of those microbes might be harming or even killing us, what about the rest? Could they be helping us heal and grow, or even think and feel? Could our microbes be in conversation with our immune system – and explain why diseases related to it are increasingly common? Or why we get cancer, what the appendix does or even why some drugs work and others don't? For the best part of two decades I have sampled the microbiomes of generous patients undergoing surgery in theatre number three, in my quest to find answers to these questions. I've also followed the work of colleagues across the world and we've shared our findings and ideas. The answers have been surprising, beyond what we could have imagined.

Today, I am driven by the idea that the rise in the collection of disabling chronic diseases has been caused, over just eight decades, by the radical disruption to the colonies of microorganisms that live in and around us. And that, in our quest to cure the world of infectious diseases, we've inadvertently created a new pandemic of non-infectious ones. This book is my way of sharing my own journey to these conclusions, and my hope is that this will start a conversation about how we can provide a different type of medicine. The true promise of microbiome science is in disease prevention.

In March 2020 I stood in the same operating theatre and watched young, previously healthy patients being ventilated for COVID-19, the disease caused by the SARS-CoV-2 virus. They were being cared for in an operating room because we had run out of space in the intensive care unit and there was simply nowhere else to put them.

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The surgeon's role was to help turn these patients on to their fronts while they lay in their medically induced comas. The aim was to redistribute the fluid in the lungs and help them breathe. It was grim work. The hospital was being overrun, and during the following weeks we sweated through our painfully inadequate PPE as more than 400 patients and members of our staff died from the coronavirus. Thousands of others suffered needlessly under harrowing conditions of enforced isolation while the government hosted parties and drank. At the time of writing, more than 670 million cases and 6.8 million deaths from the SARS-CoV-2 pathogen have been reported worldwide, reinforcing many of our deepest-held fears about the lethality of microbes. The pandemic reignited the war on bugs, a war that started with germ theory 150 years ago and mutated into a toxic mysophobia (an irrational fear of germs and contamination).

The microscopic life forms that preside over our health and wellness are increasingly frustrated with the mistreatment inflicted upon them by hyperglobalization – and they have a formidable molecular arsenal with which they are demonstrating their displeasure. The result is that although we are living longer than ever before in history, we are not living happier. Medicine doesn't have all the answers for this paradox. In response, some of my patients are now returning to ancient strategies for the treatment of their modern maladies . . .

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*Introduction: Good Sh*t*

How lucky you English are to find the toilet so amusing. For us, it is a mundane and functional item. For you, the basis of an entire culture.

Baron von Richthofen, *Blackadder Goes Forth*

After two decades of surgical training, I specialized in the treatment of bowel cancer. I spend large parts of my working day talking to patients about their bowel habits, and many of them want to talk about little else. As a nation, we British are obsessed with our gut function, largely because it has never been unhealthier. There is also a deeper, more fundamental fascination with the digestive system; the colon is a national source of comedy that has kept us going through every crisis since the beginning of time. 'Shit' is a crucial and ubiquitous word that serves as a noun, a verb and an adjective, propping up the entire English language. This wondrous word is simultaneously a profanity and a term used to denote an item of high quality, and it is liberally sprinkled into the daily chatter of our lives and even into my operating room. 'Shit! What is that?'

The sense of revulsion we feel when we're faced with human excrement (or even just the thought of it) is, in part, a response to the way it looks and smells. The brown stain is caused by a pigment called stercobilinogen, a by-product of the gut bacterial metabolism of blood, specifically haem, the iron-containing molecule that binds oxygen in haemoglobin, and the foul stink is caused by the chemical products of intestinal fermentation. But that revulsion is also a psychological reflex, ingrained by potty training and social stigma. This aversion is an important safety mechanism: hand-washing and sewer systems prevent the spread of diseases that have killed millions.

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But what if I told you that faeces was not toxic waste, and that it contained the secret to human health?

Would you eat it, if your life depended on it?

What if it was rebranded as a faecal microbiota transplant (FMT) or, more accurately, a faecal milkshake given through a tube that passes through the nose into the stomach? You could even take it in the form of a capsule – or 'crapsule' – if you wanted.

To help persuade you this might not be such a terrible idea, I'll tell you the tale of a patient. Raymond had driven the number-seven bus between Oxford Circus and East Acton from the age of twenty until taking early retirement in his mid-forties. After Ray's brother died all too young of a heart attack, Ray learned that he, too, had heart problems, which he was told were genetic. He gave up his job as a bus driver, on his doctor's orders, and used his retirement to learn about computers.

Like you and me, and everyone else on the planet, Ray was a host to several trillion microbes – the name for all living organisms that can only be seen under a microscope – that lived in and on his body. From our first breath to our last, and even beyond, microbes are our ever-present companions. While they take up residence in any number of places in our bodies, they're especially keen on – and abundant in – the various cavities and niches found in our gut. The 'gut microbiome' is the name we've given to describe not only the wildly diverse collection of microbes that live there, but also what happens when they interact with each other and with our bodies. In other words, it's an ecosystem made up of trillions of microbial life forms going about their business inside us, as we go about ours. In the last two decades, scientists from across the world have started to leverage the new science of the microbiome to transform how we conceive of human health.

Throughout his life Ray's gut microbiome had changed with him. As a child it helped his brain develop, it educated his immune system and it sustained and nourished him into adulthood. But as time passed, his genes and his gut microbiome began to do battle, causing multiple chronic diseases. His doctor prescribed an ever-longer list of medicines. Eventually Ray developed a type of

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leukaemia that left him profoundly frail. He had to rely on his wife Heather, a nurse at London's Great Ormond Street Hospital, for care.

Ray and Heather navigated his various health issues together until the day pneumonia struck. Ray became seriously unwell. When even breathing became difficult, he was admitted to St Mary's Hospital in Paddington, where he was treated with intravenous antibiotics. Without the drugs he would have died; with them, the infection was treated and he was discharged within the week. However, it was at this point that a terrible antibiotic firestorm started in his gut.

Imagine the worst-possible diarrhoea: opening your bowels more than ten times a day, incapacitating nausea, plus severe cramping pain in your abdomen, depriving you of sleep. Now imagine that you are frail, and your heart is working at 40 per cent of its normal function and your lungs are full of fluid. You can't breathe. Arthritis means you can't get to the loo in time. You are cold, clammy and profoundly dehydrated but can't drink enough to satisfy your thirst. You are soiled, but too close to death to care.

Three days after he had returned home, Ray's son called an ambulance. Ray was readmitted to St Mary's critically unwell and was soon diagnosed with *Clostridium difficile* infection (officially, this bacteria has now been renamed *Clostridiodes*). A 'hospital-acquired infection', this disease is a complication of twentieth-century medicine and an unintended consequence of Alexander Fleming's discovery of penicillin, the first effective mass-produced antibiotic, in 1928. It is a global problem that afflicts 500,000 people in the United States each year and it kills 29,000 of them.[1]

You can think of *C. diff* as a radicalized sleeper cell that waits dormant in the gut for its instructions from its antibiotic masters. In Ray's case, the antibiotic treatment destroyed the indigenous bacterial community and triggered an intestinal *C. diff* insurgency that escalated into systemic organ failure.

C. diff debilitates its host organism – the human being – by generating two toxins (enterotoxin A and cytotoxin B) that cause inflammation and destroys the lining of the gut. Inspecting the

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gut with a flexible camera passed into the bowel reveals an apocalyptic scene: massive destruction of the colonic architecture, which is left raw and bleeding. The particular strain of *C. diff* in Raymond's gut had engaged in an aggressive campaign of molecular warfare. His personal gut-microbe collection, carefully and uniquely developed since his birth and fuelled during lunches on the number-seven bus, was gone. His intestine was failing and he was dying.

The doctors at St Mary's quickly diagnosed his condition by looking for the toxins that *C. diff* makes in his faeces, and he was treated with yet more antibiotics. This seems counter-intuitive, but it is in accordance with best practice. Bacteria don't simply roll over and die, though. *C. diff* has a trick up its sleeve, which is to produce antibiotic-resistant spores that wait to germinate, biding their time. Raymond was given an antibiotic drug called vancomycin. Many patients will respond to vancomycin, but about one-quarter will relapse. And in those who do, 45 per cent will have a second relapse. These are the patients who typically benefit from FMT, or the 'good shit'.

Dr Ben Mullish, a clinical scientist at Imperial College London, was running a trial of FMT in patients with *C. diff* infections. Ray was so unwell that Dr Mullish offered him the treatment. Heather understood that there are good and bad bugs and advised her husband to go ahead with it, but Ray was not having it. The idea of taking another human's faeces was just too much for him, and he refused. Three days later, when he had deteriorated further despite the vancomycin, there was no other choice. Dr Mullish gained Ray's consent for the trial and set to work on preparing the transplant.

The logistics of preparing an FMT should not be underestimated. Faecal donors have to be found – harder than you might think. Most of us are squeamish about pooing in pots, and we struggle to do it on demand. Some studies use friends and families, others use members of staff, volunteers or 'pooled' samples taken from lots of donors mixed together. The complexity and demand for faecal transplants have spawned an entire industry, and FMT can now be purchased frozen from biobanks. Just as with any

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organ donation, donors have to be carefully screened to make sure they don't harbour transmittable diseases or parasites. Potential stool donors undergo a rigorous screening questionnaire, medical interview and examination, followed by blood- and stool-testing. Then there are the practicalities. Fresh samples must ideally be acquired within a short time from delivery, diluted with sterile saline, stirred, strained and then poured into a sterile bottle. Dr Mullish's job can at times be less than glamorous.

Once the faecal cocktail was mixed (shaken, not stirred), the transplant was administered to Ray during a colonoscopy. This procedure involves a flexible telescope that is passed into the colon through the bottom, and the bowel is gently coated in the soothing balm of microbes, which are passed through the colonoscope using a syringe.

While an FMT might be a new idea to many of us today, the medical practice of faecal transplant is ancient, and it has been drunk as 'yellow soup' since the fourth century AD for the treatment of infective diarrhoea. In 1958 an innovative surgeon, Dr Ben Eiseman, administered faecal enemas to his patients in Denver, Colorado with severe recurrent *C. diff* infections. It was remarkably effective, but like all important medical discoveries, this intervention was largely ignored at the time of its first report. More than half a century later, the Dutch gastroenterologist Josbert Keller and his team at the Amsterdam Medical Centre randomized patients with recurrent *C. diff* into three groups. The first group received vancomycin, a wash-out of the colon using a strong laxative, and a faecal transplant. The second had vancomycin and the colonic wash-out, and the third just received vancomycin. The FMT group did so much better than the other two groups that the study had to be stopped early, as it was deemed unethical to continue; 93 per cent of patients who had an FMT got better, compared to only 31 per cent with the vancomycin alone, and 23 per cent with the vancomycin and wash-out.[2]

The race to discover how FMT works is now on. We do know it restores the metabolism of bile (a green digestive fluid made in the liver and stored in the gall bladder), which is co-metabolized by

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bacteria, and this in turn blocks the germination of *C. difficile* and controls the infectious disease. It is also probable that a process of 'bioremediation' occurs, where the donor microorganisms consume and break down toxins that exist in the recipient's gut. However, there are trillions of organisms producing an infinite number of bioactive molecules, and each disease has a discrete microbiome. Therefore, it may really be a bit like turning a computer 'on and off' again; it's a complete reset of the gut's immunology software.

It's also becoming clear that samples from some donors are much more effective than those from others. These are known as 'super donors' and their faeces seems to contain a magical ingredient that makes it particularly effective. Before you don your cape and mask and rush down to your local faecal donation centre, you should also know that we don't understand why this happens, or whose poo will be most effective. Regardless, FMT is now being investigated with varying degrees of success in hundreds of trials across the globe. These include trials for inflammatory bowel disease, irritable bowel syndrome, obesity, acute malnutrition, diabetes, arthritis, hepatic encephalopathy (decline in brain function with severe liver disease), liver transplants, skin cancer, autoimmune diseases, Alzheimer's, neurodevelopmental conditions, bipolar disorder, hair loss, depression, neurodegenerative diseases and recurrent urinary-tract infections, to name but a few.

Some of these studies are extremely encouraging and offer treatments where few effective medical therapies exist. For example, FMT appears to be a promising treatment for irritable bowel syndrome, and a recent study suggests that its benefit can last for many years: 125 patients were randomly assigned to receive either 30g or 60g of faeces from the same donor or a placebo transplant containing their own faeces. Researchers not only found that the FMT improved the symptoms, but there was a lasting benefit three years after it was given.[3] The 60g group had a 71.8 per cent reduction in the severity of their symptoms compared to 27 per cent in the placebo group, with no long-term side-effects.

Unlike *C. diff*, where there is a dramatic and acute clinical change

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caused by a defined pathogen, the impact of FMT is less clear in chronic disease states, where it hasn't yet been proven that bacteria are the cause, or where we haven't defined exactly how the disease develops. As a result, at the time of writing in the UK and the US, recurrent *C. diff* is the only clinical condition for which regulatory bodies have approved the use of FMT. The bottom line (pun firmly intended) is that we don't understand how FMT works or its long-term risks well enough to start using it more widely in clinical practice. Nevertheless, because word of its incredible potential is spreading, there's a worrying growth in online enthusiasts offering back-street FMT 'cures'. I hope it goes without saying: please don't try this at home.

Ray's response to his FMT treatment was just like that in the reported literature. Within three days of receiving the microbiota transplant he was out of bed. Heather described it as a miracle.

If I've spent longer than is entirely comfortable talking about faeces, that's because FMT is a starting point for understanding the importance of the gut microbiome to human health. The extraordinary benefit of FMT in some patients has opened the clinical world to the idea that our microbes may have an important role in the causation and treatment of diseases where their involvement runs contrary to medical science.

The scale of the task is immense. The bacteria in the gut alone weigh close to 1.5 kg, they're made up of about 100,000,000,000,000 bacterial cells (that's one hundred trillion) – equivalent in number to the total number of cells that make up the human body – and they speak millions of different molecular languages.

Another major challenge in studying the microbiome is its physical distribution. The microbiome is dispersed across our bodies in different niches, each with varying total abundances of microbes. Being clear about our anatomical definitions is important. The gut is a long tube and it starts with the mouth, where the total abundances of bacteria are about 10^{11} cells (that is a hundred billion). It then passes through the gastric stomach (10^7), the duodenum (10^7), then through 5–6m of small bowel (jejunum 10^7 and ileum 10^{11}),

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1.5m of colon (10^{14} or 100 trillion bacteria) and, finally, the rectum and the bottom.

The colon is a big fermenting engine that breaks down the more complex nutrients that are not absorbed by the small bowel. So when I say 'gut', I really mean the colon, as this is where most microbes exist, unless I name another specific region. Because the small bowel is carpeted with millions of microscopic finger-like projections called villi, it has a total surface area of $32m^2$. The microbes that live at the tips of the villi billowing in the intestinal currents are different from those that live at their base, and in turn these are different from those that live within faeces. It's a bustling, chaotic, crowded and vibrant scene teeming with life. We are only just beginning to map all of the microbial life in this vast ecosystem – and to understand how it connects us to the world around us.

In this backward world, shit has become a therapy, used to replenish our delicate internal ecosystems, which are being lost as quickly as they are being discovered. Even with the impressive advances in biology, metagenomics and bioinformatics (computational biology), we might not be able to count and name all of the beneficial microorganisms that live inside us before they die out, mutate or evolve into something very different.

This book is not a history of faecal transplantation. It is a story about how we are deciphering the molecular language of the human microbiome, one of the great challenges of modern medicine. Faecal transplantation is a critical and fascinating tool that is being used to unlock these secrets.

Given that the microbiome influences almost every aspect of our lives, this story will be told in three parts. The first part takes us on a journey back in time to the birth of our planet and explores how microbes have shaped our biology, and how this profound evolutionary partnership with our microbiome connects us to our environment today. It tells the story of the microbial universe within us from the microbial big bang of our birth when we are first colonized by massive blooms of microscopic life forms, and how our microbes go on to shape our immune system, our sexual behaviour, our brain, our moods and feelings, our childhood and our

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death. It explains why our understanding of health is still defined by the breakthroughs in microbiology of the nineteenth and twentieth centuries, and why in the new world of the microbiome these frameworks are no longer fit for purpose.

The second half of this story explains how environmental forces outside our bodies are in constant communication with our microbes. It's a conversation that has changed dramatically in the past century or so, becoming increasingly fraught and stressful. Where we live, the air we breathe, the food we eat, the medicines we take – for better or worse, all of these factors influence the make-up of our microbiome. It's a global story of biopolitics, conflict, antibiotics and the medicines that have so profoundly damaged our microbiome.

The final part of this tale explains how the microbiome is shaping our future health. As big tech and big pharma become increasingly attuned to – and invested in – the extraordinary potential of the microbiome, there has never been a greater need to understand the microbes that live inside us.

We're only just starting to explain how, when and where the microbiome defines our risk of disease, but also how, when and where it sustains human wellness and happiness. The human microbiome represents the most important new therapeutic target that we have for treating the greatest threats to human life in the twenty-first century and for preventing future pandemics of pathogens. This was not only important for Ray; it is critical for all of us: without a stable and diverse microbiome, we may well lose our minds.

This book has been written in operating theatre number three during a global infectious-disease pandemic in order to spread a simple message: *microbes are not the enemy*.

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PART ONE

The Microbiome

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1. *A Library of the Known Microbial Universe*

The York Gospels are one of only a small collection of ancient Gospel books pre-dating 1066 to have survived the Reformation. They were written around the turn of the first millennium, in the scriptorium of St Augustine's monastery in Canterbury, and were brought to York by Archbishop Wulfstan around AD 1020. Genetic sequencing of the microbes that live on the York Gospels has revealed that the books contain populations of ancient bacteria that reside within their pages, and some of these bacteria originated from the skin of the Anglo-Saxon monks who wrote and read these religious texts. They also contain the destructive *Saccharopolyspora* genus, which causes a measles-like spotting of parchment that threatens this priceless historical artefact.[1]

As you turn the pages of this book, many hundreds of microbes – the name for all living organisms too small to be visible to the naked eye – will be exchanged between your skin and the paper of each page. The bacteria, fungi or viruses you share will be left as indelible physical markers of your interaction with it, pressed like dormant microscopic flowers. This phenomenon is the same for all the books that you have ever read. The quantity and diversity of the microscopic life forms found on the pages of this particular book will ultimately correlate with its popularity and how many times it is opened, folded, dropped, shared or abandoned. Not all books are treated equally, and as a result they harbour varying combinations of microbes that are typically found on human hands and bodies; Bibles are vulnerable to devotional kissing and may contain oral bacteria, while academic texts are full of sweat, blood and tears. Ancient books are bound in animal skins, bringing with them DNA and species of bacteria from different animal types, while digital books and tablets harbour their own collection of microorganisms

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that can be liberally shared with anyone who chooses to swipe a finger across their screens.[2]

Using tools such as microscopy, culturing and chemical dyes, skilled eighteenth- and nineteenth-century scientists discovered that our hands have two dominant populations of bacteria that could be responsible for contaminating a book. 'Resident' microorganisms typically live on the surface or just under the epidermis of the skin, in stable colonies that predictably reproduce. 'Transient' microbiota only colonize the superficial layers of the skin and only occasionally multiply. These are the bugs you remove when you wash your hands, and they are the usual culprits that spread infection or mark the pages of a precious manuscript.

While classical microbiology has transformed our understanding of how microbes known as pathogens cause infectious diseases, it doesn't tell us how intricate communities of microbes from different species or kingdoms work together to cause disease or maintain our health. In other words, it doesn't provide information about how bacteria, fungi and viruses communicate *among themselves* or *with you* to maintain healthy skin, or how the dynamics of these populations change under shifting environmental pressures of your daily life, like using soap, wearing gloves or stroking a pet. Collectively, we spent US $2.95 billion in 2022 on hand sanitizer and, although this reduces rates of pathogen transmission, it is fundamentally changing the health of our skin microbiome in ways that are not well defined or understood.

Microbiology makes way for microbiome science

If every piece of paper has its own microscopic living history, providing an indirect record of human behaviour and clues about our health, then each book also has its own microbiome: a living ecosystem defined by the genes from combinations of bacteria, yeasts and viruses and the environmental conditions needed to maintain their existence. Each page of a book has its own tiny niche of microbial species, seeded from the hands of the person who read it, contaminated by microbes

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acquired during eating or touching and sampled from the environment of whichever part of the world the book was read in. Think about that the next time you pick up a novel at the airport or train station and displace it in a foreign land.

Microbiome science is a departure from classical microbiology. It wants to understand how communities of microbial organisms support human life or cause disease. By studying the genomes, habitats and environmental conditions of all living microorganisms residing within a niche over time, it provides a measure of the collective functions of these interacting communities. A microbiome scientist looks at a library of books and sees a vast ecological time-capsule, where the bacteria of skin from ancient hands and mouths can still be found on the covers and pages of texts, giving us clues about the people who wrote and read them. From this perspective, your bookshelf can be thought of as an ecosystem that's unique to you. The choice of titles has been shaped by your economic, social, ethnic and biological status and these same powerful forces also influence the microbiome dotted throughout your collection. The microbiome can be studied at the level of the library, shelf, book or page.

Humans, too, have a number of ecological niches, or microbiomes. These are distributed across our anatomical landscape and organs such as the lungs, skin and urogenital tract. The largest collection of microbes lives in our gut, where their quantity equals the total number of human cells found within the body. No corner of the body is sterile all the time, and, just as on the skin, the colonization by microbes of our most important organs can be transient or persistent. Our different microbiomes may also have varying functions, which will change depending on the environmental pressures they are placed under. In general, our symbiotic bacteria (bacteria that live in a close mutualistic relationship with us) are important for our health, but defining quite how important isn't straightforward. We know we can live without some of them some of the time – we don't die every time we use the bathroom, and patients who lose their colons during surgery can live quite happily. But a true microbial niche implies a sophisticated co-dependence between

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microbe and human, in a mutually beneficial relationship that has developed over evolutionary timescales. This means that some of our organs have adapted to host these tiny passengers, and that our microbiomes influence both our risk of chronic diseases such as cancer and our well-being. However, because the human microbiome is so large, dynamic and variable between people, determining *who's there* and, perhaps more importantly, *what they are doing* is a huge challenge.

Microbial dark matter

In the early 1930s, while poring over his peers' recent observations of the Coma Cluster of galaxies, the Swiss astronomer Fritz Zwicky noted something strange. According to the measure of visible mass, galaxies were moving too quickly for the cluster of planets and stars to remain bound together. Or, to put it another way, they were not generating enough gravity to hold them together, and by his calculations these galaxies should have shattered. Zwicky suggested that a previously unidentified form of mass that he called *dunkle Materie* (dark matter) might explain this observation.

Dark matter consists of invisible, undetectable particles that influence everything within the cosmos. It explains how stars move within galaxies, how galaxies pull on each other, and how all that matter got clumped together in the first place. Dark matter is ubiquitous and essential for life; it is unseen but felt in every corner of the universe.

Its opposite counterpart is 'dark energy', which describes a force that appears to be driving the expansion of the universe. If Zwicky's dark matter holds the cosmic flesh in place like an invisible skeleton, then dark energy continually tries to pull it apart.

Dark matter and dark energy are not explained by what's known as the 'standard model', which defines how elementary subatomic particles make up all known matter. The standard model says nothing about the 95 per cent of the universe that physicists believe is not constructed from normal matter.

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Modern medicine is similar in this respect: we have a comprehensive model that explains a large part of the known mammalian biological processes that we can see, and which we use to explain the majority of the diseases we experience. But the standard model of medicine fails to account for the majority of genomic data in the human body, which we can't yet see.

You can therefore think of these microscopic life forms within us as a sort of biological dark matter. We only have a superficial knowledge of how they promote health and bind together the spinning human galaxies of interconnected organs. These organisms also produce dark energy, a chemical and immunological force that sustains us – and that will ultimately destroy us, if we allow it to become unregulated. The human cosmos feels the effect of microbiome dark matter and the energy it produces every day. Because twentieth-century medicine couldn't see it or measure it, its influence on human health has until now been attributed to the components of our biology that can be determined by human genes and measured by X-rays, CT scans and blood tests.

From human to metahuman

Beyond the protection of important texts, a librarian's main job is to maintain order. This is a complex task, once achieved through an intricate and laborious process of manual indexing and labelling. That was until the late twentieth century, when computing transformed the speed and accuracy with which these tasks could be performed. The same processes have dramatically influenced how evolutionary biologists index all life on Earth.

In 1837, Charles Darwin scribbled out a picture of a tree, an illustration that he used to explain the evolutionary relationships between species. In this metaphor, all life on Earth grew from a single organism, and the major divisions (or branches) on his tree could be divided into animals, plants and 'protists'. Protists were microscopic organisms that couldn't be neatly packaged into the animal and plant divisions. This tree underwent many modifications,

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and by the 1990s it was assumed that all living organisms could be divided into just two main evolutionary branches or 'domains' of life: prokaryotes and eukaryotes.

A eukaryote is an organism with complex cells, or a single cell with complex structures that exist within it. In these cells the genetic material is organized into chromosomes, which are safely stored within the cell's nucleus. You are a eukaryote, but so is a fungus, a plant and a polar bear.

Prokaryotes are organisms that lack a cell nucleus, and this is the domain of life where bacteria live. You may think of fungi and bacteria as very similar inconsequential microscopic organisms, and it would seem logical that they are related. But an evolutionary biologist would argue that you have more in common with a fungus (your fellow eukaryote) than a bacterium does, because fungi and bacteria are from different domains of life.

Each of these domains divides again into kingdoms and, within each kingdom, there are six further major divisions: phylum, class, order, family, genus and species. Each describes a shared anatomical detail or physiological function, and the microbiome can be described at each of these divisions, before it branches into vast clades, or groups of species.

Perhaps the greatest biologist never to win a Nobel Prize was a man named Carl Woese. In 1977 this revolutionary made a discovery that would shift and expand our understanding of the evolution of the natural world, which would re-draw the Darwinian tree-of-life model.[3] His genius was to adopt a strategy of defining the similarity of species not on the basis of their morphology (anatomy), physiology or metabolism, but on their genetic code. Instead of using the newly discovered DNA, he chose a molecule called ribonucleic acid (RNA). One of RNA's many jobs is to make proteins and, to do this, specific types of RNA bind to a cellular machine called a ribosome. Woese and his team managed to elucidate the structure of ribosomes and work out that small sub-units of ribosomal RNA are highly conserved between species – an amazing feat when you consider the staggering number of ways the ribosome can be folded and created. Within a single bacterium called

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Escherichia coli, for example, the potential variations in ribosomal structures are larger than the total number of elemental particles in the entire universe.[4]

Because comparatively small regions of the ribosomal RNA are discretely conserved between species, it can be quickly and affordably analysed to identify organisms based on their genomic signature. With this innovation, Carl Woese and his collaborator George Fox identified a third domain of ancient microscopic life, as distinct from bacteria as bacteria are different from plants, animals and you. They called this domain the archaea, and it comprises some of the most ancient microbes on the planet. Their varied and exotic characteristics allow them to survive in conditions that should be impossible, and some archaea are known as 'extremophiles'. For example, *Methanopyrus kandleri* can grow in temperatures up to 122°C and can survive in atmospheric pressures 200 times greater than those found on the surface of the Earth. You literally couldn't boil it or crush it to death. *Ignicoccus hospitalis* thrives on hydrothermal vents on the bottom of oceans, and other species of archaea can be found in acid mines and in caves that have never seen light.

Collectively, archaea play a key part in maintaining the biogeochemical health of the planet, and they are also an integral part of your microbiome.[5] These extremophiles are well adapted to live in and on us, and archaea have been found in the human gut, mouth, vagina and on the skin. Archaea such as *Haloferax massiliense* are able to live in high salt conditions and they help us metabolize a Western diet, while other species play an important role in removing heavy metals. They also help maintain intestinal health by producing the gas methane; these archaea are known as 'methanogens' and account for about 10 per cent of all anaerobic microscopic life forms that live in the gut. Methanogens in the intestines of our livestock, and those that live in our sewage systems, oceans and wetlands, are also shaping our climate, and they have contributed to a 0.50°C rise in our planet's temperature increase since pre-industrial times.

Carl Woese's work provided a 'metataxonomy', or a microbial ribosomal RNA gene inventory, from which all life on Earth could

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be indexed. This decoupled evolutionary biology from the science of biological organization, and it laid bare the extraordinary diversity of bacteria and archaea. For example, one recent phylogenomic analysis demonstrated that of the 135 described phyla across the three main kingdoms of life, 104 belong to bacteria, twenty-six exist within the domain of the archaea, and the eukaryotes – the domain where you, I and every other animal species reside – possess a paltry *five*. Estimates of the actual number of bacterial and archaeal species living on our planet now range from millions to billions.

If you're wondering where viruses fit into this system of organization, the answer is that they don't. Viruses are obligate cellular parasites that borrow the machinery they need to replicate and survive from their hosts, which means they are not technically 'living'. They exist instead within a separate 'empire' to other cellular life forms – one that's also defined by its incredible diversity.

By the end of the twentieth century the race was on to unlock the secrets of DNA. The human genome was finally mapped, at a cost of nearly US $3 billion, by the Human Genome Project (HGP) in 2004. At its most basic level, this discovered that a human is coded by approximately 20,000 'genes' composed of precise strings of base pairs that code for proteins made by RNA. The human genome is remarkably stable between people, and even between species. You and I share 99.9 per cent of the same genes, and in turn we share 98.9 per cent of these genes with our closest evolutionary relatives, such as the chimpanzee.

But the Human Genome Project forgot something crucial: its gene library was figuratively covered in microbial life forms. In this new 'metahuman' model of the internal biological universe, the human genome makes up less than 1 per cent of the genetic code that influences our health and well-being, and the remaining 99 per cent originates in the community of microbes that live within us, known as the 'metagenome'. Unlike the human genome, this is massively diverse between individuals. People from different families or parts of the world will share less than 10 per cent of species that reside in the gut, and even less with a chimpanzee.

Nevertheless, mapping the human genome turbocharged the

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