Chapter 2
of

Contrary Life and the Technical Fix
from malaria vaccine to hormone contraceptive

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Chapter 2

Diabetes: synthetic insulin faces the wider world

The boy slowly wriggles and kicks within his mother, eager to be born. But first he has a more serious task to complete. Out in the open world lurk everywhere the worst threats to his life: microbes and parasites. These can harm or kill him more surely than any flood, drought or snake, unless his own developing defences against them are strong.

In the core of his bones, holding pulpy red marrow, are vast numbers of blood cells growing from their primordial stem cells – simple cells destined for various tasks. Some of them will mature into the predominant red cells with their single task of transporting oxygen around the infant's body. Or they will mature into the translucent wisps of living matter called white cells. For the boy they have much work to do, despite a slight genetic flaw he has inherited and which might gradually manifest itself in a few of these white cells.

The tasks of the white cells are both simple and a maze of complications. They find, slow, stop or kill invaders; from miniscule viruses to revoltingly large worms. But the types and varieties of these invaders would fill a telephone directory with their forms that shift, alternate and evolve rapidly as the microbes reproduce. So the problem for the white cells is how to prepare for the onslaught of such pathogenic invaders before the infant suddenly is exposed to them on his day of birth. Some of his bone marrow cells will mature into the first line of defence, an innate general barricade of macrophage and neutrophil white cells that are alert to anything that resembles a microbe as something potentially dangerous. Microbes succumb to engulfment and digestion by these cells. In turn, the microbes respond by not resembling microbes. They hide within tough outer capsules, they hide behind ever changing outer coats of protein, or they penetrate rapidly to the inside of cells where they can hide – even inside white cells.

Other cells will mature into a line of defence that can learn from experience. They will acquire specialist armaments, continually adapting to what types of microbe attempt invasion. But how to learn before being born, how to learn before being exposed to a world teaming with microbial and parasitic threats to health and life? The infant's genetic code provides a mechanism both delicately subtle and cumbrously profligate.

White blood-cells, of the type of known as lymphocytes, develop as the baby boy grows. They either mature into cells dedicated to secreting soluble antibodies into the blood or tissues to inactivate or kill microbes – the B-lymphocytes. Or they will become T-lymphocytes that directly face up to microbes and kill them with secreted enzymes or by inducing bursts of toxic forms of oxygen. A very particular mechanism within the genetic code of the stem cells sets about generating, at random, thousands upon thousands of different detectors for microbes. These are molecules out on the surface of the cells. They act as receptors, each with a unique

and precise shape and arrangement of charges to lock automatically onto some specific protein likely to be on the surface of a microbe. A microbial key and its one and only lock on an individual lymphocyte. There are many microbial keys, or antigens, and as many or more lymphocyte keys. As the white cells mature further they will be tuned so that the entire repertoire of receptors is multiplied immensely, 10 followed by 16 zeros of separate capacities to detect then fight off new invaders. Not an infinity of receptors, infinity is a big concept indeed, but a number ample to tackle all likely threats. A mechanistic generation of chance, a mechanism that itself arose by natural selection of chance mutations, thus acts as a roulette wheel endlessly spinning out receptor molecules of exquisitely exact defensive fit to foreign threats of similarly random origin.

The B-lymphocytes prepare themselves in a simple way compared to T-lymphocytes. The latter migrate from the marrow of the boy's long bones to his thymus gland. This small organ lies in front of his heart and specializes in the maturation of lymphocytes in a second stage generation of anti-microbial receptors. The mature T-lymphocytes bear an additional mechanism of immune signalling: arrays of molecular antennae all over the outer surface of the cell by the name of the histo-compatibility complex. All cells bear this system for compatibility of tissues unique to individuals. Conversely, they can detect and signal anything that is non-self. This provides a large variety of specificities for different lineages of T-lymphocytes. Coupled to those in turn are the receptors for many thousands of foreign antigens. So what develops in the boy's thymus and spreads to his lymph nodes and spleen are millions of different capacities to recognize and attack invaders of all varieties. Clearly the body can only accommodate a small number of individual lymphocytes bearing each unique lock; they continue as small reserves waiting their call to action.

The boy's immunity is developing in a way that looks ready to face the world, but for one fundamental problem. Within his growing fluids, cells and tissues there are many proteins exposed to antibody and T-lymphocytes. Some of his own proteins can appear to the blindly mechanistic molecular receptors of immunity as mere antigens that need to be dealt with – inactivated or attacked. Already his mother's immunity has adapted to presence of her son as not wholly of herself; the genes of the boy's father gave rise to that anomaly.

The gargantuan task of the boy's developing immunity is to select out and rid itself of every single lymphocyte with any chance character of being a self-attacker. As the bone marrow churns out lymphocytes by the million, so the other organs of immunity discard them by the million. A ruthless process, called apoptosis, will weed out any lymphocyte that is not properly tolerant of the boy’s tissues. The marked lymphocytes are induced, by mechanisms for suicide they contain, to shrivel, die, and disintegrate. The debris is engulfed and digested by the all-scavenging macrophages.

Every cell of the body starts out with a fundamental drive, hidden within its DNA, to divide into two, and again, and so on forever. The boy started as a single cell, a primordial egg in his mother's womb. Now he comprises one hundred trillion cells.

They have been marshalled and directed to create the shape and function of a human body by a complex array of signals, checks, and stops by apoptosis. His hands and feet first grew as simple undifferentiated buds expanding at the ends of his limbs. Fingers and toes were then sculpted from the soft flesh by the chisel of apoptosis to create the spaces between the five digits of each limb.

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Growing up amongst northern pine forests and lakes, enjoying the seemingly endless days of brightly lit summers, the boy grew healthy and resilient to the assaults of microbes and of the cold dampness and lack of fresh fruit and green vegetables of the long winters. His particular genetic peculiarity lay hidden; he was healthy as long as it did not encounter and act in concert with some other chance factor from the outside world.

In the autumn of his fifth year the boy felt ill with stomach upset, vague malaise and lethargy. Yet another microbe was at him – an enterovirus this time, invading first his gut from contaminated food, then travelling deeper to the liver, pancreas and other organs. His immunity fought and won the acute battle, but the ensuing guerrilla war left pockets of infection in his pancreas. Many of the cells of this small organ, which sits next to the massive liver, are dedicated to the job of controlling the supply of energy rich molecules to every organ and cell within the body. Molecules of glucose dissolved in the liquid plasma of blood, or conversely molecules of insoluble glycogen stored in the liver and muscles.

Insulin is the controller molecule – a hormone created within and secreted by the beta-cells of the pancreas. As the boy digests his breakfast his brain becomes more alert as the supply of glucose increases and his muscles tremble in eagerness to kick his football around the garden. But excess glucose is potentially harmful to his body, so insulin acts to store most of the flood of glucose from his digestion of breakfast. The store is glycogen held in muscle and liver. By the time the boy's midday meal is approaching, the glycogen store is being mobilized for conversion into glucose to maintain the level in the blood. Another hormone, glucagon, does that balancing job after being secreted by the alpha-cells of the pancreas. Too little glucose and the boy will collapse with lethargy; too much and the tendency of glucose to react with natural enzymes will damage his body.

The very cells that produced insulin are themselves as finely balanced between the opposing demands of the body's internal fires as is the balance that insulin maintains. The beta-cells are peculiarly susceptible to damage by those forms of oxygen known as free radicals, typically nitric oxide or hydrogen peroxide – potent weapons, the daggers and pistols of the immune cells. Every cell of the body has to guard against self-inflicted wounds by the protection of special anti-oxidant enzymes. The chemical fire-fighting role that evolved in the beta-cells seems to have left them exposed to dangers of combustion and structural mayhem. They are more susceptible to oxidative damage than other cells.

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An enterovirus invading a beta-cell is bad for the cell, but the combination of immune defences against the virus and normal growth and replacement of beta-cells keeps the body healthy. Unless there is a weakness in the immune defences, unless the specificities of the histo-compability system of the boy lack something that renders some of his lymphocytes intolerant of beta-cells, especially any beta-cells infected with a virus.

Macrophages that circulate in the blood and the lymphatic system are alerted to the invading virus in the pancreas and are attracted to remain there. Foci of these infiltrating cells of innate immunity slowly build up around the many clusters of beta-cells within the pancreas, causing chronic inflammation. Reactive radicals of oxygen from the inflammatory cells stress the beta-cells, upsetting their normal delicate balance. In this state the beta-cells begin to appear as non-self in this boy with his abnormal histo-compatibility system. The beta-cells appear to be covered with antigen keys that will fit the locks on those lymphocytes expressing the abnormal receptors.

Some of the T-lymphocytes in the broad classes known as CD4 positive and CD8 positive acted in concert against the boy's own tissues. The CD8 cells started to behave as blind and remorseless killers of beta-cells. Onto beta-cells alone they released tiny but focused bursts of poisons: nitric oxide and hydrogen peroxide, and granules of enzymes. Beta-cells died in a perverted form of apoptosis; scavenging macrophages mopped up the remains. Immunity turned against its own body as an autoimmunity. Cell by cell the pancreas lost beta-cells faster than new beta-cells could be produced. The constant balancing act between glucose and glycogen became short of insulin. The boy began a swing back and forth, daily and hourly, between the dangers of excess and insufficient glucose. By the time he was ten years of age his doctor diagnosed diabetes; the type 1 version of the condition, dependent on level of insulin. The doctor prescribed insulin and taught the boy's parents how to help administer it and to adjust his diet to lessen the burden on the balancing system. The insulin, however, was essential. Without it the boy's life would have been short, with it he still enjoys a normal and productive life.

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The weight of microbes on Earth is more than half the weight of all the plants, and of course the weight of plants must greatly exceed that of all the animals as mere herbivores, carnivores or parasites. Microbes outnumber animals and us humans overwhelmingly but only a tiny percentage of the types and species of these microbes can invade and infect humans. Nevertheless we are constantly surrounded by them, breathing them in, ingesting them, occasionally having them injected by bloodsucking insects. The threat of disease and death from these ubiquitous pathogenic microbes is constant and greater than predators and natural disasters combined. Our remote ancestors faced a similar threat from microbes hundreds of millions of years ago. Long before there was any hint in the animal world of creatures that would eventually evolve toward humans there were animals such as the sea sponges and the terrestrial insects with well developed immunity against

microbes. Plants possess immunity of a kind, they can defend themselves against invasion. No immunity – no chance of survival.

Moreover, without an ability rapidly to evolve new immune defences against new variant microbes, survival of animals would be short lived. This never ending race between attacking microbe and defending immunity, illustrated by malaria in chapter 1, is known as Red Queen Effect. The queen is a character in Lewis Carroll's surreal story of *'Alice through the looking glass . . .'*, she is a living chess piece who must keep running fast just to stay in the same place.

Textbook authors conventionally describe our defences against microbes as the immune *system*. Unfortunately there is little about immunity that is systematic. A medical instrumentation engineer reading a typical account of immune defences of humans, or a hospital administrator wandering into a seminar on the topic – people who design and operate systems that generally work as intended – would be bewildered by the bizarrely contradictory, overlapping, redundant and even dangerous mechanisms of immunity. More anarchy than system.

An evolutionary biologist, on the other hand, approaching immunity for the first time would likely recognize it as an accumulation over many millions of years of separate responses to invasions by an endless variety of type, strain or species of microbe and parasite. Each response succeeded sufficiently to improve the chances of that human to survive and reproduce better than other humans without that particular new response. The responses were evolved adaptations determined by what the genes made possible through variation and inherited onwards. Unsuccessful responses, or lack of response, would by definition result in death or lower reproductive success. Constant threat of death or debility from infectious diseases was the grim condition of humanity until the discovery, just several generations ago, of the very existence of microbes followed by the invention of anti-microbial treatments and informed supportive medicine. In the days before vaccination or antibiotics, infection with one of the various bacteria or viruses causing meningitis, inflammation of the membranes around the brain, was common and most likely fatal; and so on for a long list of dangers.

As with immunologists and their system, there remain biologists clinging to a concept of perfection of evolved organs and animals. The perfect vision of the eye of a hawk that can see, from a great height, the twitch of a blade of grass that betrays the presence of a mouse, or even see the ultraviolet light returning from a streak of urine of the mouse. The anatomical facts tell a different story. The vision of a hawk is better than that of a human which is much finer than the best camera, but like all backboned animals we have eyes that no camera designer would accept. Incoming light has to pass through a layer of blood vessels then a layer of nerve fibres before reaching the layer of sensory cells. In practice this matters little, but from the human concept of design it is a mistake of evolution. The eyes of an octopus or squid do better, they correspond to what the inventors of the first cameras designed, with the sensory layer presented to the light first. Evolution has come up with at least forty separate types of eye; some are better than others but all do a job sufficient for better survival. So, by some tiny random mutation eons ago

that determined one pathway rather than an alternative, hawks make do with an optically poor design of eye because to switch to a better one would take them as long as the evolution of the first design.

Evolution is blind, it blunders here and there as tiny new developments derived from changes in the genetic code of an individual, from mutations sparked spontaneously or by outside influences such as natural background radiation or toxins from wood smoke. Our coldly indifferent physical environment and our surrounding hostile microbes, parasites and predators select useful changes and weed out non-starters. Our eyes, useful for finding food and evading predators, evolved from clumps of cells on the skin of some worm-like ancestor. They evolved in response to a single phenomenon of physical nature – photons of radiation of a small range of wavelengths, a stable and unvarying character of our physical environment. Our eyes, imperfect but adequate, are a fine biological system of many types of tissues forming the lids, lens, iris, muscles, vitreous gel, blood vessels, nerves, sensory layer and of course a large part of our brains to perform the seeming miracle of converting the energy of photons into nerve impulses that our brain can use to see the ripening fruit in the trees. The eyes of our ancestors that first began to walk upright were scarcely different from ours. All humans born fully healthy now start life with vision systems that are functionally identical.

Our immune defences evolved in response to an immense number of types and species of pathogenic microbes, each of them capable of endless and rapid variation and the ability to evolve into something new far faster than we can evolve. Human peoples who have been exposed for millions of years to the microbe that causes the deadliest type of malaria, the *Plasmodium falciparum* of tropical Africa, are born with better immune defences against that specific microbe than peoples from parts of the world where the microbe cannot survive. More than that, we all have immune defences that are anticipatory; they can acquire new abilities in response to new attacks. My immune defences are different today than one month ago before I was invaded by yet another new strain of the common cold virus; in a month's time the defences will change again after exposure to the new strains of influenza virus in the vaccine I will receive.

The inherited ability to acquire immunity to killer pathogens by getting artificially vaccinated is wonderful but it comes at a price. The problem with acquired immunity is that the only things the body has to fight microbes with are its own cells. Like a bare-knuckle fight – potentially as dangerous to defender as to foe. The balance between killing non-self cells of the invaders and killing self-cells of the pancreas is delicate. As an evolutionary path the balance works for most of us most of the time. That criterion has been good enough for evolution in humanity's long past; for the practice of medicine that balance tipping the wrong way is a dire problem.

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In a laboratory of the University of Oxford, Dorothy M. Hodgkin was given a small batch of crystals of natural insulin. They entranced her with the idea of working on a substance of such importance. She ignored the immense difficulties of revealing their three dimensional structure. As a structural biologist she deciphered the way in which the constituent atoms of huge molecules of proteins were connected and folded into the basic operational units of all living things. The year was 1934, she was twenty four years old and fresh from tutelage by one of the masters of the art at Cambridge – John D. Bernal. By 1969, with the structures of cholesterol, penicillin and others along the way, members of her research team were ready to publish their account of the full atomic structure of insulin in three dimensions.

Meanwhile, in the University of Pittsburgh, Panayotis G. Katsoyannis had a team working fast to devise a way to synthesize insulin chemically. They published their first report on this in 1966. Katsoyannis stated clearly that they had based their synthesis directly on information published in a series of papers between 1949 and 1951 published by Frederick Sanger at the University of Cambridge.

Sanger had adopted insulin for the same reasons as Hodgkin: because it was an important protein and readily available in pure form. A self-effacing man of intricately skilled and creative biochemical method, he naturally chose a pragmatic approach – two dimensions would provide the blank sheet on which he planned to draw a diagram of how all the building blocks of the molecule, its amino acids rather than its atoms, were joined in sequence to constitute the primary structure of the molecule. A quiet researcher maybe, but of highest ambition: this had never been done before. He invented his own unique methods for the task, entirely separate from those used by the structural biologists. For a long time scientists had misunderstood proteins – pure chemists remained disdainful of their messiness and apparent irregular amorphousness.

Sanger planned to invent a fresh analytic approach. He demonstrated that proteins comprise strings of amino acids linked in precise sequences that are unique for each protein and upon which their specialist function depends. In the discussion sections of his papers he mentioned his hope that the knowledge he and his colleagues had provided would someday be useful; the same hope as expressed by Dorothy Hodgkin. Neither of them imagined the strange ways their wishes would be fulfilled.

This story contains many recursive loops, although fewer than in a molecule of insulin, and we need now to glance at one of them. Insulin, so prized as a research material, was discovered as a natural hormone by Nicolae Paulescu working in the Bucharest of 1916. A difficult place and time for such an endeavour, so it was almost inevitable that others would scoop him of the main fame. The University of Toronto hosted the team of Frederick G. Banting, John J.J. MacLeod and Charles H. Best. Many authors have told their story – revealing much about the devious routes to discovery and invention. Here it is sufficient to tell that in the Toronto General Hospital in 1922 James B. Collip resuscitated a boy of fourteen years from a terminal diabetic coma by injecting insulin that he had specially purified for clinical use. The drama of that salvation and the many that soon followed

reverberated through medical clinics worldwide. The early insulin, isolated from the pancreases of cattle or pigs from the slaughterhouse, was difficult to purify but the pharmaceutical firm Eli Lilly and Company of Indianapolis rapidly developed methods. They soon produced large supplies of pure natural insulin for use in diabetic wards and dispensing by physicians.

Meanwhile, Panayotis Katsoyannis patented a method for the chemical synthesis of insulin by means of creating and stringing together amino acids. However, his method required two hundred separate steps, making it too expensive to compete with the natural product despite its advantage of being intrinsically free of potential agents of infection. Furthermore, this story concerns a wholly different approach to producing insulin: cheaply, in large quantities, without use of dead animals, thus able to meet increasing demand despite a relatively declining supply of the natural product.

To continue with the story of that amazing technical fix we need to return to Dorothy Hodgkin. Her monumental life's work on the full atomic structure of insulin had no direct connection to any of these three routes to medical insulin, but her indirect impact was crucial. Hodgkin, Bernal, Max F. Perutz, Rosalind E. Franklin, John T. Randall, Maurice H.F. Wilkins, to name the most conspicuous in just Britain, formed a school of structural biology that led a deliberately cultivated new way of doing biology. Physicists and chemists, equipped with powerful machines and mathematical intelligences, joined in studies of the protean, messy complexity of things alive. They peered deep into crystals of protein with X-ray machines, custom-made to focus their intense radiation onto single crystals. They determined, after processing of vast indirect data, the positions of individual atoms relative to each other in three dimensions. X-ray crystallography they called it, and the school was strongest for a while within the golden triangle of Oxford, Cambridge and London.

The main challenge was the structure of proteins, but a few other researchers were entranced by the even deeper problem of the nature of the genetic material. Was the information that had to be contained within the nucleus of each cell encoded in proteins, as seemed likely for such complex molecules, or was it held in the simple and rather boring nucleic acids, the DNA and RNA? As evidence from geneticists working with cultures of microbes increasingly pointed to the nucleic acids, so structural biologists realized that DNA had to be investigated. William T. Astbury was the first bold outsider to try, at the University of Leeds; then Linus C. Pauling focused his formidable resources onto the delicately thin threads of semi-crystalline DNA held in tiny glass vials.

The art of X-ray crystallography in those days, before computers and robotic apparatus was a craft indeed. Requiring the manipulative skill of a brain surgeon, the mental capacity of a mathematician to perform Fourier analysis by hand, and the patience of the sort of saint that Dorothy Hodgkin became, it took decade after decade to accumulate knowledge. Understanding it all was a problem on a higher plane still.

This is where James D. Watson and Francis H.C. Crick make a cameo appearance in our story. Using the crystallographic data of Rosalind Franklin and the technical insights of Maurice Wilkins, these two outsider theorists cut through the maze to the glowing prize of discovering how DNA works. The researchers who soon gathered to admire the tottering scaffold of rods and plates that was the literal model of the molecule, in a scruffy room of the Cavendish Laboratory in Cambridge, were awe-struck by the intellectual beauty of how the function derived so directly from the simple structure.

The concept of evolution by Charles Darwin and Alfred Wallace became endowed with exact knowledge of a precise molecular mechanism for replicating the information needed to grow organisms. By the time the revolution of molecular biology was underway, the evolutionary biologist George C. Williams and others were developing a gene-centred interpretation, to be popularized by Richard Dawkins's startling concept of selfish genes. They penetrated through to a deep understanding of life by reducing their analysis to the simplest minimal units: genes as the replicators. Anyone in search of any vital essence of life would probably find it only in these replicators.

For Crick and Watson the structure and function of DNA was the quick and seemingly easy part of their lifetime achievements. For the obvious question next was how does the vast information contained in the tape-like structure of DNA, a fantastical molecule of length measured in metres, translate into molecules of protein. How is the information passed, starting from the single nucleus in the one-celled egg that initiates a human embryo, to the enormous number of molecular building blocks called proteins to build a full human being?

These two stories of discovering how DNA works, followed by the desperately hard collective struggle by a hundred or more researchers over a decade to understand how DNA informs protein synthesis have been well told. To come back to our route to a new kind of insulin we need to jump to the mid 1960s at the University of Geneva. There geneticists Werner Arber and Daisy Dussoix used the microscopic workhorse of many laboratories, the gut bacterium *Escherichia coli*, together with a type of virus that infects them. Parasitism is such a common way of making a living that it should have surprised nobody to discover that bacteria suffer viral infections. The ditty about big fleas having 'little fleas upon their backs to bite 'em and so on ad finitum' was apt notice. Such viruses are called bacteriophages, literally eaters of bacteria. The Swiss researchers discovered that their *E.coli* bacteria were able to defend themselves from infection by bacteriophages. The mechanism of what seemed to be a primitive form of immune defence presented an open challenge.

At Harvard University, Matthew S. Meselson and colleague Robert Yuan were in lead position to respond. Meselson had earlier collaborated with Francis Crick and two other stalwarts of the protein coding problem: Sydney Brenner and François Jacob. The pair now at Harvard had used *E.coli* and a type of bacteriophage as tools in one of the key experiments of that era so they were then well placed for the mental somersault of thinking of bacteria as having their own immune capacity.

Soon they found in their bacteria an enzyme that conferred the ability to resist, or restrict as they termed it, infection of bacteria by bacteriophage. The enzyme was of a type known as endonuclease, acting directly on the nucleus of an individual bacterium. This particular restriction endonuclease cut through the backbone of DNA whilst leaving intact the groups, the nucleotide bases, that carry the genetic code. This enzyme is a pair of molecular scissors.

From a laboratory in Washington University, St. Louis, Arthur Kornberg had already discovered other enzymes, ligases, able re-join these cut bonds in DNA. An enzymic toolkit combining exquisite finesse with enormous power was beginning to emerge from the various researchers who had rushed to corroborate the discovery of Meselson and Yuan. A question fermented in the imaginations of many of them now that they knew that restriction enzymes could be used to cut out interesting fragments of DNA and that these fragments could be spliced to others of interest. Researchers expected the combined section of DNA, if assembled with intent, to retain the full sequence of codes for the amino acids to build a protein. This understanding traced back to the first model of DNA in which the code comprises four units, the nucleotide bases, which complement each other as two strict pairs. Two cut ends of DNA with complementary pairs placed together will reunite automatically.

So the question resolved into a transformational proposition. Can we take the gene for an important protein, a hormone say, and somehow insert it into a bacterium or yeast cell so that the new host is tricked into manufacturing the protein? Will we be able to extract it in a form that we can sell for medical treatments?

Paul Berg was the right man, right time, right place. He worked in Arthur Kornberg's lab on the action of amino acids and had gained that strategic position via a route from early studies in biochemistry at Pennsylvania State University – interrupted by service as a pilot in the US Navy – followed by research on cellular enzymes for a doctorate from Western Reserve University. For an experimental workhorse Berg chose a microbe called Simian Virus 40. Originally, researchers found this as a contaminant in their cultures of cells derived from monkeys that they used for studies on vaccination against polio. Berg's enthusiasm for this hardworking laboratory microbe grew so much that by the time he moved to California he ordered a personalized registration plate for his Honda automobile, reading just SV40. Enormous potential as an experimental tool resided in the ability of this virus to infect human cells without causing any obvious harm. Berg and his colleagues dubbed it a cloning vector, in plain English a carrier of copies of DNA. Berg was typically forthright about his new mission: the practical goal of genetically based therapies for people using the products of the new science of molecular biology.

David Jackson, Robert Symons and Paul Berg, then at the Stanford University Medical Center, were first to demonstrate the technique that would soon become known as recombinant DNA technology, and a little later in popular speech as genetic engineering. They started out with the circular DNA of SV40. They cut it to produce linear strands and onto these they inserted genes from a particular

bacteriophage and also the cluster of genes of *E.coli* that code for the enzyme that splits the sugar galactose. They then closed the strands to circles. What they had produced was the equivalent of an unusual but natural form of DNA known as a plasmid. It contained the precise genetic information to code for a specific protein, the enzyme for galactose that they could detect and assay experimentally. Could they then place the plasmid in an organism that could actually produce the protein?

In 1952 Joshua Lederberg had introduced the term and concept of plasmid to bring together various descriptions of unusual units of inheritance that had been found occurring naturally in bacteria. He clarified that plasmids are self-reproducing pieces of DNA, in circular form, that can transfer between individual bacteria of the same species. They are not essential to the normal life of the bacteria. They do, however, contain the information used by bacteria to produce enzymes that can destroy antibiotics. In practice such plasmids can transfer antibiotic resistance to the strains of bacteria that happen to possess them, greatly to the selective advantage of such bacteria if they are assaulted with antibiotics. What was becoming a huge problem for clinical doctors was about to become a huge opportunity for molecular biologists.

Who might now be in in place first to grasp the chance? Stanley N. Cohen at Stanford University, Palo Alto, and Herbert W. Boyer at the University of California, San Francisco, met for the first time in 1972 at a conference in Honolulu. As soon as they realized they needed each other they met over a late night snack at a bar – a meeting of such portent that it is now commemorated by a statue. They found they were in position to prove that plasmids assembled in a laboratory could function when they inserted them into *E.coli* so that the bacteria would be induced to hijack the mechanism for protein synthesis of their new host. Cohen knew how to manipulate plasmids naturally occurring in *E.coli* to distinguish them by their resistance to the antibiotic tetracycline. Boyer had recently discovered two different restriction enzymes in *E.coli* and knew how to handle them to cut DNA.

On return to California, working in labs at either end of the busy San Francisco peninsular, they got to work on combining the properties of their particular plasmid and enzymes. Boyer's enzymes cut DNA in a precisely crucial way: now a chisel cutting a mortise joint so that the two strands of DNA are left exposed and the staggered ends become complementary to any other similarly staggered end of DNA that possesses the matching pairs of nucleotides. Cohen and Boyer invented gene splicing.

The resulting new piece of nucleic acid was a clone, that is the original piece of DNA could be replicated as an exact copy of itself by dividing again and again within bacteria. The researchers took a new plasmid from *E.coli* that codes for an enzyme conferring resistance to tetracycline antibiotic. They inserted this into a strain of *E.coli* that normally could not resist tetracycline. The desired result for this crucial experiment was proof that the new host bacteria had become resistant. When it worked they knew they had demonstrated the possibility of transferring the capacity to manufacture a specific protein, innate to one species, into another

species of organism. The second organism would then produce the protein within itself. Cohen and Boyer invented recombinant DNA technology.

Boyer became famous enough by 1981 to see his face printed on the cover of *Time* magazine. The painting is respectful whilst cleverly revealing a combination of an elderly cherub and a youthful Albert Einstein – complete with a shock of dark wavy hair and walrus moustache. Nevertheless, Boyer had at college played football as a linesman. As an independent researcher in San Francisco he had struggled for money to support his obscure probings into the life of bacteria whilst suffering banishment to a decrepit lab by his colleagues who feared his microbes might contaminate their own cultures. He came from a background in Pittsburgh where his relatives and friends typically found jobs in mining and railroad businesses. Assigned to give a presentation in his small liberal arts college where he had enrolled on a pre-medical course, he chose DNA as his topic. This novel molecule so fired his imagination that, as he put it to author Stephen Hall, he thought to himself 'To hell with medicine. Who needs all these sick people to take care of? I want to do something interesting.' Boyer understood that these tiny but tough bacteria not only replicate very fast, they will also do so within industrial size vats if supplied with a simple broth of nutrients. Each of the trillions of bacterial cells would constitute a miniature factory for a useful protein if the inserted genetic material coded for the protein. Money could be made.

Stanley Cohen was another east coaster who gained his education in medicine at the University of Pennsylvania. At that meeting in Honolulu he came to understand the potential of genetic engineering but he never fell in love with the money making potential of it. Notwithstanding such reservations, soon Cohen and Boyer worked out how to transfer pieces of DNA using their gene splicing into species of vertebrate animals as well as bacteria. The time looked ripe to push this technique up to the bizarre level of a manufactured symbiosis between human medicine and bacterial genetics. Working in the team with Boyer was Francisco Bolivar, of the University of Mexico. He led the authorship of a pair of papers first to demonstrate that artificial plasmids could be constructed for cloning in bacteria for practical purpose. First the genetic information for a medically important human protein would be characterized and assembled, that would then spliced into an artificial plasmid, bacteria would be infected with this plasmid, the bacteria grown in bulk, and finally the desired protein would be extracted from the bacteria and purified for clinical use. Cohen and Boyer patented these methods as soon as possible. They meant business, but what would be the best human protein to grow in their bacteria?

At a meeting devoted to insulin held 1976 in Indianapolis and sponsored by the Lilly company, the hot topic was gene splicing as a route to a new form of insulin. Then, insulin was one of Lilly's major lines, with up to eighty five percent of US sales. Despite that, the company felt nervous their position would be weakened because the supply of pancreases from slaughterhouses was failing to meet demand from the expanding number of people in America with type 1 diabetes. The predominant form of diabetes, type 2, was also affecting more and more people, and insulin can also be useful in the treatment of that separate condition. Crisis was

predicted in twenty years time. Lilly had already considered the chemical synthesis of insulin and steered away from its expensive complexity. As alternative they started to investigate the commercial prospects for insulin made through the recombinant DNA route.

Many molecular biologists from laboratories throughout America were consulted, but the executives from Lilly were disappointed to find that support was limited to a few research groups and pessimism prevailed. The ethos and developing tradition of molecular biology about the mid 1970s was for pure research into the fundamentals of how genes work at the deepest molecular levels. Grant money was needed for this of course, but few researchers considered making their own money by moving from discovery to invention to selling products. The Eli Lilly Company hedged its bets by a loose involvement with Boyer, another group in California, and Walter Gilbert over at Harvard.

Herbert Boyer knew he must force his own route past the negative responses he received from the commercial companies he had approached. He needed capital to set up in business – he needed a financial business partner. By the luck that comes to those searching it, Boyer was approached by Robert A. Swanson, a venture capitalist working for a firm in California. Swanson had independently identified synthetic insulin by the recombinant DNA method as a path to high profits. He had tried an obvious company in California, without success. With Boyer the response was dynamic despite the unlikely match of personalities. An early photograph of the pair, once they were in business, shows them side by side in front of a mass of biotech plumbing. The younger Swanson is business suited in grey with white shirt and tie whilst Boyer wears a pink open-neck shirt, leather waistcoat and blue jeans. Both look confidently pleased with their success.

Swanson was a New Yorker who had studied chemistry then business management at Massachusetts Institute of Technology before starting a career with Citicorp Venture Capital, then moving to Kleiner & Perkins in San Francisco. He was twenty eight years of age, with little capital of his own but impatiently ambitious. He intended to set up a full research, development and manufacturing company based on what he was convinced would be the burgeoning field of molecular biology. The vital first decision would be what products to offer for sale. Any product had to be suitable for a simple and rapid passage through tortuous regulatory requirements. It had to be easy to sell to a large and wealthy market because they needed, as a start-up company, to avoid what the pharmaceutical business calls missionary marketing. Selling drugs for little or no profit to needy customers for reasons of philanthropy and favoured company image was strictly for the big boys. Insulin was an obvious choice. Without delay Swanson and Boyer created the company Genentech, both staking $500 on it.

For Boyer, the next problem was what precise method to select for actually constructing the gene for insulin. One possibility was to use a chemistry kit approach of putting together the DNA entirely synthetically. Alternatively a biological approach could be used based on the natural process of the transfer of information from natural DNA to protein via the intermediate nucleic acid,

messenger RNA, then make a copy of the natural human gene, resulting in a gene for insulin consisting of complementary DNA. The chemical method that Boyer chose was more difficult and little tried compared to the well known and reliable biological method.

He was prescient, basing his decision on fear of the adverse publicity and paranoia already sprouting around genetically modified organisms. About that time this was creating enormous problems for some molecular biologists, especially over on the east coast. A political circus arose and the researchers made strenuous efforts to regulate their own work, but the trend overshot. Walter Gilbert and colleagues at Harvard for example, found that to comply with the new regulations they were obliged to experiment in a laboratory with the grade of microbiological safety precautions appropriate to contain the most dangerous microbes to human health. They ended up experimenting inside the lab of the British government then known as Porton Down, originally established for investigations on warfare germs. The complex logistics of their transatlantic endeavour confounded their experiments. Boyer meanwhile, because his chemically synthesized DNA genes for insulin were not technically of human origin, was able to squeeze past these paranoid barriers. At least there seemed to be no regulations specifically forbidding his chemical method.

In 1976 Boyer contacted colleague Arthur Riggs at the City of Hope National Medical Center, at Duarte up against the hills on the edge of Los Angeles, to ask if he was interested in collaborating on synthetic insulin. Riggs replied he was preparing a grant for work with Keiichi Itakura on a similar approach to synthesize the gene for the hormone somatostatin, then insert that into bacteria for mass production as a clinical drug. For the pair at Duarte this would be a trail run, working with a small and simple protein, leading toward synthesis of more complex proteins with large markets. Somatostatin, a protein of fourteen amino acids compared to fifty one for insulin, regulates the workings of human growth hormone. Absence of somatostatin leads to gigantism, a rare problem. Natural human somatostatin can only be obtained from cadavers. Riggs, however, was having trouble with potential funders who regarded their preliminary project as implausibly ambitious. Boyer, despite the misgivings of Swanson about this diversion, decided that Genentech should support this project instead; he felt that their new company needed the highly specialized skills of Riggs and Itakura.

Thus a powerful team assembled, focused on a two step route to their goal. First demonstrate capability with somatostatin, then rapidly move on to insulin by inventing and patenting both, prior to setting up for manufacture and sales. Roberto Crea, David Goeddel, Herbert Heynecker, Dennis Kleid and others were typical of the production workers at the laboratory bench. Highly qualified, mostly research fellows on the post-doc treadmill of meagrely paid short-term contracts. Feverishly they worked crazy hours set as much by the physiological demands of their microbial cultures as by their own ambitions to make a name for themselves and thereby get a proper job.

By then DNA based methods were sufficiently advanced that nucleotide bases for the chemical synthesis of DNA were commercially available, extracted from salmon sperm which consists mainly of cell nuclei, sold by the pound weight for modest prices. The bases had to be joined to the sugar containing groups, the nucleosides, to form the full nucleotides. The process is basically simple but the reactions never go to completion, so each step had be purified separately to remove the remaining reactants that would otherwise spoil the next stage. Then the nucleotides had to be strung together as triplets that would, when in the bacteria, code for synthesis of the strictly related amino acids. Somatostatin required forty two nucleotides for its fourteen amino acids; far more for insulin. Finally these nucleotides had to be formed into the classical double helix structure of DNA. The patents of Itakura and Riggs, filed in 1979 and granted in 1982, defined the technical and legal basis for synthesizing both somatostatin and insulin via microbial cultures. For insulin the patents referred directly back to the information from Frederick Sanger and colleagues in the early 1950s on the amino acid sequence of the natural hormone.

The dawning revolution in biology spread its light to big commerce and high finance when Genentech issued 1.1 million shares in October 1980 at $35 each. By an hour later the selling price rose to $89. By the end of trading that day the price settled down to $71. A good day's work for the owners and staff of Genentech – $35 million net, then unprecedented in the history of Wall Street.

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Insulin, purified and ready in a vial for injection is a superbly successful technical fix. Insulin synthesized by the recombinant DNA method is a substantial improvement on the original natural product. Far more than that, the rapid uptake of this new insulin opened the door to a revolution in technology now coming to rank alongside the revolution in electronics brought about by transistors. The next protein to be synthesized in this way was the source of the new vaccine against hepatitis B, the troublesome natural antigen of the vaccine, derived from blood of people infected with the virus, was replaced with a factory-made pure version to enormous benefit. The anti-malaria vaccine uses a recombinant antigen.

When researchers solved the problem of the natural synthesis of proteins and inventors followed that with artificial synthesis, biology transformed into a science that could be done as chemistry. Instead of the fickle, poorly predictable variability of living things, best studied with large samples analyzed statistically, a huge area of the study of living things was opened up to the ability to handle different molecules in precise amounts and relative proportions with exact expectations of what the resulting new combination of molecules would be. Literally test-tube biology. The potential new power of biotechnology far exceeds the old ways of plant and animal breeding or the extraction of natural products. This entire revolution derived directly from research into the fundamental questions of what is the nature of the genetic material and how does its nature inform the construction of proteins. Nobody engaged in this pursuit of pure knowledge and understanding had in mind any specific practical application. Not until the early 1970s did a few

researchers realize that the deep understanding and range of new techniques could possibility be combined for the synthesis of medical proteins.

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