Chapter 2
of

Contrary Life and Technical Fixes
from
malaria vaccine
to
hormone contraceptive

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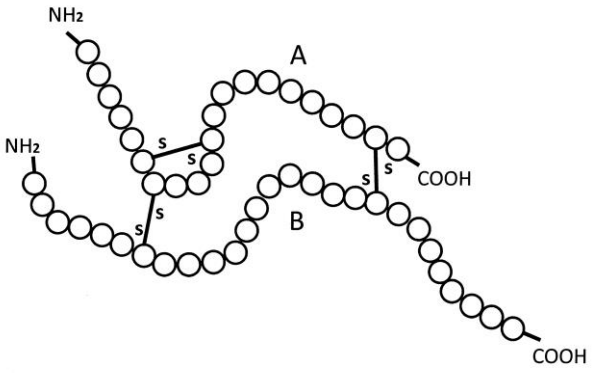
Diabetes, autoimmunity and synthetic insulin



The boy slowly wriggled and kicked within his mother, eager to be born. But first he had a crucial task to complete. Out in the open world lurked the worst threats to his life: microbes and parasites. These would harm or kill him more surely than any flood, earthquake or traffic accident, unless his own intrinsic defenses against them developed strongly.

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

The tasks of the white cells were both simple and a maze of complications. They had to find, slow, stop or kill invaders; from miniscule viruses to revolting large worms. But a list of types and varieties of these invaders would fill a library with their forms that shift, alternate and evolve rapidly as the invaders reproduce. So the problem for the white cells was how to prepare for the onslaught the fetus would suddenly be exposed to when born.

Some of his bone marrow cells would mature into the first line of defense, an innate general barricade of macrophage and neutrophil white cells that were alert to anything that resembled 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 rapidly penetrate inside human cells to hide. Some microbes even hide inside white blood cells.

Other cells would mature into a line of defense that can learn from experience. They would 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 fetal boy's genetic code provided a mechanism both delicately subtle and cumbrously profligate.

White blood cells of the type known as lymphocytes started to develop as the fetus matured in his mother's womb. They originated from stem cells in the marrow of long bones. Two basic types of lymphocyte grew there: B-lymphocytes and T-lymphocytes. The B-lymphocytes matured fully within the bone marrow to become cells dedicated to secreting soluble molecules of proteinaceous antibody into the blood or tissues. These antibodies would directly inactivate foreign toxins or kill microbes exposed in the blood or tissues. The T-lymphocytes first migrated from the bone marrow to the thymus gland, situated beside the heart, to complete their maturation. The T-lymphocytes would directly face up to cells infected with microbes, or to cells that are cancerous, and kill them with bursts of secreted enzymes or with highly reactive forms of oxygen.

In both these types of lymphocytes a very particular mechanism within the genetic code of the stem cells set about generating, at random, thousands upon thousands of different detectors for microbes. These were molecules out on the surface of the cells. They acted as receptors, each with a unique and precise shape and arrangement of charges to lock automatically onto some specific protein. A protein likely to be on the surface of a microbe. A microbial key and its one and only lock, specific to an individual lymphocyte.

There were many microbial keys, or antigens, and a nearly matching number of lymphocyte locks. As the white cells matured further they would be tuned to increase their diversity so that the entire repertoire of receptors was multiplied immensely, to at least ten billion 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. Here operated a mechanistic generation of chance, a mechanism that itself arose by natural selection of chance mutations. It was a roulette wheel endlessly spinning out receptor molecules of exquisitely exact defensive fit to foreign threats of similarly random origin. And since the boy's body could only efficiently accommodate a small number of individual lymphocytes bearing each unique lock, they continued in him as small reserves waiting their call to action.

Every cell of the body bears an array of molecular antennae all over its outer surface. These molecules are glycoproteins that span the cell's plasma membrane. They are genetically determined by genes of a group known as the major histocompatibility complex. This compatibility system of a cell signals to the immune system that the cell belongs to that genetically unique individual. It signals that the cell is self rather than non-self as an invading microbe would appear. As proteins of the cell are continually turned over, small fragments, peptides, are carried to the cell surface by new MHC molecules. At the surface the MHC molecules present the peptides as epitopes. The epitopes are areas of the peptides potentially capable of stimulating an immune response, they are potential antigens. Self epitopes present a bland, harmless appearance to the body's immune system. But if a cell contains degraded proteins from an invading microbe, some of the presented epitopes will be non-self: they will alert the immune system.

The T-lymphocytes respond to these non-self epitopes. Bound through the plasma membrane of the T-lymphocytes are large molecules in a double form, consisting of two different chains of protein, as a heterodimer. This protein complex is the T-cell receptor. These receptors come in various basic molecular formations and within them is an immense variety of receptivities to different epitopes on antigenic molecules.

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The boy's immunity was developing in a way that looked ready to face the world, but for one fundamental problem. Within his growing blood and lymph liquids and his cells and tissues there were many proteins exposed to antibody and T-lymphocytes. Some of his own proteins could appear to the blindly mechanistic molecular receptors of immunity as mere antigens that needed to be dealt with: inactivated or attacked. Already his own and his mother's immunities had both adapted to the presence of his fetal body as immunologically non-self. The boy shared equally the genes of his father and mother. Not only was he genetically different from his mother, he was as genetically unique as every other human.

The gargantuan task of the boy's developing immunity was to select out and rid itself of every single lymphocyte with any chance character of being a cell that could attack other ordinary cells of its body. He had to purge his self destructive cells. As the bone marrow pumped out lymphocytes by the million, so the other organs of immunity discarded them by the thousand. A ruthless process, called apoptosis, weeded out any lymphocyte that was not properly tolerant of the boy’s tissues. The marked lymphocytes were induced, by natural mechanisms for suicide that they

contain, to shrivel, to die, and finally disintegrate. The debris was engulfed and digested by the all-scavenging macrophages.

This removal of non-tolerant lymphocytes had to be ruthless. Every cell of the body had started from its origin as a dividing stem cell with a fundamental drive, hidden within its DNA. They were driven to divide in two, and divide again, and so on forever. The boy started as a single cell, a primordial egg in his mother's womb. Now was constructed from trillions of cells. They had been marshaled and directed to create the shape and function of a human body by a complex array of signals, checks, and stops by this implacable force of 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. Meanwhile his particular genetic peculiarity lay hidden; he was healthy as long as it did not encounter and act in concert with some 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 immune system fought and won the acute battle, but the ensuing guerrilla war against what remained of the infection left pockets of virus 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 and ready for action, or conversely molecules of insoluble glycogen stored in the liver and muscles.

Insulin is the signaling molecule – a hormone synthesized within and secreted by the beta-cells of the pancreas. As the boy digested his breakfast his brain became more alert as the supply of glucose increased and his muscles trembled in eagerness to kick his ball around the garden. But excess glucose was potentially harmful to his body, so insulin acted to organize storage of most of the flood of glucose from his digested breakfast. The store was glycogen, held in muscle and liver. By the time the

boy's midday meal was approaching, the glycogen store was being mobilized for conversion into glucose to maintain the level in the blood. Another hormone, glucagon, did that balancing job after being secreted by the alpha-cells of the pancreas. Too little glucose and the boy would collapse with lethargy; too much and the tendency of glucose to react with cellular enzymes would damage his body.

The very cells that produced insulin were themselves finely balanced between the opposing demands of the body's internal fires. The beta-cells are peculiarly susceptible to damage by those forms of oxygen known as free radicals, typically nitric oxide (nitrogen monoxide) and hydrogen peroxide. These are potent weapons, the daggers and pistols of the immune cells. So 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 defenses against the virus and normal growth and replacement of beta-cells keeps the body healthy. Unless there is a weakness in the immune defenses, unless the specificities of the histocompability system of the boy lacked something. Possibly some of his lymphocytes could be intolerant of beta-cells, especially any beta-cells infected with a virus.

Macrophages that circulated in the blood and the lymphatic system were alerted to the invading virus in the pancreas and were attracted to remain there. Foci of these infiltrating cells of innate immunity slowly built up around the many clusters of beta-cells within the pancreas, causing chronic inflammation. Reactive radicals of oxygen from the inflammatory cells stressed the beta-cells, upsetting their normal delicate balance. In this state the beta-cells began to appear as non-self. The beta-cells appeared to be covered with antigen keys that would fit the locks on those lymphocytes expressing receptors for the boy's abnormal epitopes.

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, hydrogen peroxide, and granules of proteolytic enzymes.

Beta-cells died in a perverted form of apoptosis; scavenging macrophages mopped up the remains. Immunity turned against its own body: 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 to swing back and forth, daily and hourly, between the dangers of too much glucose or insufficient glucose. By the time he was ten years old his doctor diagnosed diabetes; the type 1 version of the condition that is 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 insulin the boy's life would have been short, with insulin he continued to enjoy 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 because all animals, at the end of their food chains, depend on plants for food. Microbes, being tiny, thus outnumber animals and us humans overwhelmingly. 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, and occasionally having them injected by bloodsucking insects. The threat of disease and death from these pathogenic and ubiquitous 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 immune defenses of a kind. No immunity: no chance of survival.

Moreover, without an ability to evolve rapidly new immune defenses against new variant microbes survival 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.

Textbooks of immunology start by reference to a system called immunity, then continue with dense descriptions of a bewildering maze of overlapping, redundant, contrary and even dangerous mechanisms. Immunity can seem more anarchy than system. However, from the perspective of how a system might have evolved, immunity reveals itself as an accumulation over millions of years of separate responses to invasions by an endless variety of microbes, parasites and toxins. In parallel the

immune system has responded to an endless succession of varieties of tumor cells that must be destroyed before they turn cancerous. Each effective new defense improved the chances of the human in which it occurred to survive and reproduce better than other humans without that defense. The defenses were evolved adaptations determined by what the genes made possible through variation and were inherited onwards. Unsuccessful defenses would, by definition, result in death or lower reproductive success. Constant threat of death or debility from infectious diseases and cancer was the grim condition of humanity until the discovery, all too recently, of antibiotics, antivirals, and cancer drugs and treatments.

The amazing inherited ability to acquire immunity against invading killer pathogens or tumor cells running amok comes with a heavy price. The problem with acquired immunity is that the only weapons the body has to fight 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, as a working compromise, the balance works for most of us most of the time. That compromise has been good enough for evolution in humanity's long past. But for the practice of medicine the balance tipping the wrong way creates 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 the 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: especially because it was a protein readily available in pure form. A self-effacing man of creative

biochemical method, he chose a pragmatic approach. Two dimensions would provide the blank sheet on which 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.

Sanger 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 and we need now to divert to 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 endeavor, 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, which reveals 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 specifically 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 collected at 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 for 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 of them working just in Britain, formed a school of structural biology that led a deliberately cultivated new way of doing biology. For a long time scientists had misunderstood proteins – pure chemists remained disdainful of their protean messiness and apparent irregular amorphousness. But now physicists and chemists, equipped with powerful machines and mathematical intelligences, joined in studies of the complexity of living things. 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 at the California Institute of Technology 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 lab 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 fabulous 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 how function derived directly from structure. So simple, so beautiful.

For Crick and Watson the structure and function of DNA was the quick and 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 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 defense 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 rejoin 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 who knew that restriction enzymes could be used to cut out fragments of DNA and that these fragments could be spliced to others. 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 automatically reunite.

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 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 lead to recombinant DNA technology, or in popular speech 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 make 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 would 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 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 like 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 fast, they will 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, lots of it.

Stanley Cohen was another east coast man 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 and the bacteria grown in bulk. 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 Eli 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 American 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 is also useful in treatment of that separate condition. Crisis of insulin supply 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 was plentiful. The prevailing 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 University.

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 favored 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 endeavor confounded their experiments – their efforts ran out into the sand. 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 induces 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 meagerly 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 secure 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 were required 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 on October 14, 1980 at $35 each. After one hour the selling price was $89. By the end of trading that day the price settled to $71. A good day's work for the owners and staff of Genentech – $35 million net, unprecedented in the history of Wall Street.

♦

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 ranked alongside the revolution in electronics brought about by invention of 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 similarly 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. Previously biologists were limited to the fickle, poorly predictable variability of living things, best studied with large samples analyzed statistically. Then a huge area of the study of living things was opened 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.

Nevertheless, a mismatch haunts the role of insulin in care of people with type 1 diabetes. Insulin worked as an early treatment despite many large gaps in knowledge and understanding of how type 1 diabetes starts and then of how the immune defenses malfunction so as to end up attacking the beta-cells of the pancreas. The pancreas of humans is hidden from view, inaccessible, not amenable to culture and mimicked best by diabetic mice. The difficulties of studying the precipitating causes and immunity of type 1 diabetes are like the astronomy of the dark side of the Moon.

The condition is increasing as numbers of new cases diagnosed each year in a large sample of people, that is the incidence of the disease. Insulin, of any sort, is a technical fix without influence on this unhappy fact of public health. It is a treatment, not a preventive, curative or eradicator. In contrast, hepatitis B is reduced in incidence by the new recombinant vaccine because the replication rate of the single specific cause, the virus, is reduced and thus transmission to susceptible people is reduced in a virtuous positive feedback. Furthermore, better public health hygiene helps that reduction.

The causes of the increases in type 1 diabetes range from increased environmental pollution to increased transmission of enteroviruses caused by lapses in hygiene and even to the bizarre extreme of too much hygiene. As an example of what we face in handling the increase in type 1 diabetes this possible problem with hygiene goes like this. Humans evolved with multiple immune defenses against a wide variety of microbes and parasites that have been trying to infect us for millennia. One of our advantages against the external bloodsuckers at least is the combination of bare skin, dexterous fingers and the grooming of infants by parents and close relatives. Bathing

and changes of clean clothing helped improve that original state and the invention of soap and sewage disposal greatly reduced attack by microbes and internal parasites. Modern evidence based medicine with vaccines, anti-virals and antibiotics now provides many of us the privilege of living our lives entirely inside a protective bubble that keeps at bay the great majority of our historic living threats. Airborne contagious microbes like the bacillus of tuberculosis and the virus of influenza are the predominant threats that remain difficult to reduce and avoid.

This hygiene hypothesis claims that now the natural maturation of any one person's immunity in response to repeated assaults from many different types of pathogens has become faulty through lack of stimulation. As if the power of the immune defenses have insufficient to occupy themselves against, they get up to self-harm. An army yearning for action that inadvertently starts fighting amongst itself.

The proposed mechanics of this malfunctioning process of immune maturation remain controversial. There is much circumstantial evidence, and experimental evidence is necessarily indirect. My point about the hygiene hypothesis is a single example of the wide context of causes and prevention of type 1 diabetes. The specific and magnificent achievement of medical insulin, synthesized by the recombinant DNA process, provides sufficient leeway and respite to enable continuation of the struggle to hold back the increase in this condition. Therapy using regulatory T-lymphocytes is one route that has to be explored further. Some kind of immune stimulation to diminish the probable influence of infection with an enterovirus is another. There remains much to be done in a wide and interacting social and technical context. Meanwhile, more insulin will be needed for a long time into the future. Just as well that it can now be conjured up out of a combination of chemicals and cultured bacteria as an industrial factory product.

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