Chapter 1
of

Contrary Life and Technical Fixes
from malaria vaccine to hormone contraceptive

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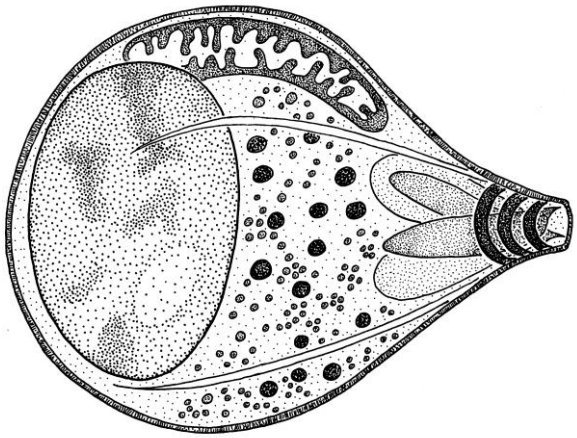
Malaria: set a vaccine to catch a parasite



A child floated on waves of drowsiness, dipping in and out of sleep as if subconsciously aware of some danger on the night air. Laden with vapors, the air spread moisture from the recent rains and wafted odors of cooking and wood smoke through a pervading blossom scent and the fustiness of rot. Vibrating on the air were incessant croakings of frogs, bursts of laughter, scraps of music from a radio. The people of the village attended their evening chores, social business and pleasures. Thunder clashes rolled southward from beyond the nearby border, somewhere beyond the edge of the Congo River basin.

The child tried to sleep under the bed-net. The bed, pushed up against the wall, made room for the charcoal cooker, boxes of food and clothes, a table, two chairs and a radio: her home. Later she would share bed-space with her mother, but at four years she now often found herself balanced at the edge, with an arm pressed against the net's fine meshwork. Up the wall wafted a tremulous plume of air, the child's breath and sweatiness, slightly warmer and moister than the rest. The plume oozed between the top of the mud wall and the thatch of the roof to join the other odors of the night.

On the wing nearby was a mosquito. Sensing extra carbon dioxide in the plume, the mosquito followed the clue up through gaps in the tattered thatch, then down around the plume with its shimmer of warm odors rising through the bed-net. Blood is what the mosquito sought, another meal after laying its third batch of eggs onto a nearby puddle. Hunger for nutrients to supply another batch of eggs drove the mosquito before it would die of exhaustion, or was eaten by a bat. Only blood would do, particularly that of a human, and the child's skin against the net was little barrier to the finely flexible tube of the bloodsucker's mouthparts. As the mosquito

thrust and probed around in the skin, seeking a minute capillary blood vessel just below the surface, it squeezed out saliva to ease the probing and prevent clotting of blood.

The hint of danger on the air manifested here, in that dribble of insect saliva in the child's skin. Microbes, single celled protozoan animals of the plasmodia kind, slithered away from the pool of saliva and out between the fibers and cells of the skin. These plasmodia also sought blood, but instead of the cost of minor irritation from the bite of a mosquito, this bunch of just eighteen plasmodia could multiply over and over again to vast numbers of blood seekers teeming within the child.

Within the mosquito, in its gut then salivary glands, the plasmodia had developed to their stage called sporozoites. Miniscule threads of living matter, they had a central nucleus containing the genetic information for the rest of their development, and an array of organelles at the forward end called an apical complex for penetration into individual cells of the child. Moreover, they contained a mitochondrion organelle for energy transformations, and another organelle called an apicoplast which, like the mitochondria, was derived from an ancient microbe that came to live together with another precursor cell. Altogether a new and sinister combination cell that now sought to live within yet more cells, to live off them at their expense.

Even the containing membrane of the sporozoite, the outermost layer of the cell, was more like an organelle than a wrapper. First it acted as a molecular conveyor belt, using molecules of a protein related to thrombospondin that moved along the level of the membrane in one direction, gliding the sporozoite forward in the opposite direction over tissues within the skin. These special molecules connected to an internal arrangement of molecules that ratcheted back and forth upon a sort of chain, connected in turn to the gliding molecules – a molecular clockwork. The sporozoites sought a blood capillary in the skin. Contact with one was detected by interaction between another type of molecule in the outer membrane, the circumsporozoite protein, and characteristic molecules on the surface of these minute blood vessels. Once gliding along the outside of the vessel, the sporozoite activated its penetration apparatus to invade rapidly the blood within the capillaries. In less than a minute the sporozoite set out toward its first important home: the liver.

Eventually the child drifted into deep sleep, leaving the sentinels within her body to sound warning of the invaders. Speed became crucial for the plasmodia. Already some of the original group of sporozoites lost their way in the barrier of the skin, with its dense packing of collagen fibers. Between the fibers is a sparse film of liquid: the lymph that spreads from the plasma of the blood, past the cells and tissues of the body and drains into the ducts and nodes of the lymphatic system. Within these nodes lay many of the sentinels of the child's immunity,

distributed all over her body and already bearing traces of its defenses against previous infections with this *Plasmodium falciparum*.

Carried in the liquid of her blood and lymph were molecules of antibody specifically dedicated to this and only this species of parasite. They bound with and coated the invaders with a molecular layer that tightened around some of the sporozoites and caused them to falter and shrivel. These dying invaders were spotted easily by scavenger cells, the macrophages, within the skin and deeper within the lymphatic system and blood. These cells were equipped with wide veils of their outer membrane that could spread out and around such invaders as sporozoites, sensing them by their coating of antibody as not being of their own kind, not the self of the whole child's body. Antibody and macrophages saw the sporozoites as antigenic, consisting of foreign molecules. Sporozoites were also seen that way by special sentinel cells described as dendritic because of their long tentacles covered with a range of molecules sensing, antenna like, for invaders.

The macrophages and dendritic cells were sensitive to the minutest variations in the antigenic character of invaders, and although these sporozoites were *Plasmodium falciparum* they were not the usual variety, the kind that had been circulating between the people of the village for years. These sporozoites seemed to be something new. Any of the skin's macrophages and dendritic cells that had contacted this group of sporozoites would have become activated to detach from their posting and migrate through the lymph ducts into the nearest lymph node, the one in the child's armpit. There they would start urgent communications between immune mechanisms, presenting molecular evidence that this infection was not another usual one of the local plasmodia. In contrast, the infection was something to which a new response was urgently needed, adapted specifically to this invader.

The ability of the child's immunity to coat some of the sporozoites with antibody resided in memory cells deep within her lymph nodes and spleen. These were the archive keepers, the recorders of previous battles with this parasite. They came from a small population of cells, white cells of the blood called lymphocytes. These originated from just a particular type of cell, a stem cell for lymphocytes, deep within the marrow of long bones. They were a clone that had proliferated vastly when that one stem cell, one of billions of its kind, happened to match precisely to the character of this new antigen. The match was molecular, derived from a precise mechanism that generated random mixing of genetic elements. Once the lymphocyte's lock encountered and engaged its antigen key, the lymphocyte became activated to divide and divide into an army of immune defenders. After the battle they settled down to a stable small reserve of cells with the memory of how to multiply again to fight the same battle. An immune capacity was acquired specifically by exposure to infection.

Were these plasmodia new to the village, were they recognizable by subtly different forms of their clothing? Some of the villagers earned a living working in

the copper mines away in the east. On their journeys home they often had to spend the night in rough shelter or shacks, exposed to the mosquito species *Anopheles gambiae* – a bloodsucker specialized by long adaptation to feed on humans. The miners stay for a while in their home village and in their blood may hide a new form of plasmodia, one that has adapted to different conditions, perhaps to survive treatment with different drugs.

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The new sporozoites that remained in the child then plunged into a stormy world. Blood squeezed along the meandering channels of the capillaries, back into veins, then heart and arteries, leading back to organ and tissue. A wild and dangerous ride for the sporozoites, now surrounded by traces of antibody that might bind to them, dodging the circulating macrophages and avoiding capture in the immune filters of the lymph nodes and spleen.

Only the liver would provide respite for the sporozoites. This massive organ is dedicated to processing blood for its load of nutrients from the intestines and as filter against any product of digestion that is unsafe, from miscellaneous microbes to the alcohol in a villager's brew of beer. The liver consists mostly of biochemical factory cells, or hepatocytes. The blood gets to the hepatocyte liver cells along passages called sinusoids.

The first sporozoites arrived in the liver within a few minutes of escaping from the skin. Now they were close to a good home, a place where they could settle down to mature and produce offspring. But first they had to run the gauntlet of a fearsome array of more macrophages. These ones were so particular to the liver they go by the name of the scientist who first discovered them – Kupffer cells. Like any good sentinel cell they extend their protean forms out into the blood channel of the sinusoids to catch particles that may be a threat as they float by in the blood. The lining of the sinusoid is studded densely with these Kupffer cells, seemingly a stout barricade between the sporozoites and the homely liver cells.

As soon as the sporozoites entered the liver sinusoids the circumsporozoite and thrombospondin type molecules on their surface recognized a type of molecule, particular to the sinusoids, which coats the structural cells of these passages. The sporozoites promptly alighted here and started to glide again. Once moving this way they became poised for another traversing maneuver, using their penetration apparatus. What they traversed were the very cells that should have been trapping and engulfing them – the Kupffer macrophage cells. This audacious subterfuge took the sporozoites directly down beneath the lining of the sinusoids.

When they emerged beyond the inner side of the Kupffer cells they continued their penetration into liver cells. They had just bored, unharmed, through a type of cell dedicated to killing them. Worse was to come. As the sporozoites traversed the Kupffer cells they shed much of their outer molecular coats. These

coats then suppressed the ability of the Kupffer cells to send out warning signals to the rest of the organs of immunity. The Kupffer cells could still secrete into the blood and lymph one of their possible signals, a soluble material called interleukin-12, but only weakly now. So the assaulted Kupffer cells lapsed into a state of immune dampening.

The mechanics of immunity involves multiple interlinked, overlapping parts of many origins, with redundancies and even competitions, within individual cells let alone the entire system. The liver helps to balance the various functions of immunity. The balance is delicately dynamic, but handled well by the multi-tasking liver. This organ controls much of the base level of inflammation resulting from exposure to so many threats from the intestines.

Having evaded or switched off some cells of this formidable Kupffer barrier, the sporozoites then sought an individual hepatocyte liver cell that best suited their need for a safe hiding place. They traversed several liver cells in quick succession, with little damage left in evidence. But something about the surface coat of a particular cell changed the behavior of the sporozoites. No longer did a single sporozoite crudely pierce through the outer membrane of this cell. Instead it precisely aligned its penetration apparatus against the cell's membrane to trigger formation of a hollow in the cell membrane. The hollow deepened into a cup, inviting a single sporozoite. From the sporozoite's penetration apparatus were secreted molecules that added to the cell's membrane, and the two different outer membranes zippered up tightly together, engulfing the sporozoite within the cell. As the sporozoite descended, the membrane of the cell folded over its tail, and the cup closed over leaving the surface smooth again. Inside the liver cell was now a balloon structure, a parasitophorous vesicle, made of its own outer membrane. Within the vesicle sat the sporozoite.

For the sporozoite this could be a dangerous place to be. There were other small vesicles in the ground substance of the cell, in its cytoplasm. These vesicles could fuse with parasitophorous vesicles containing an invader to release their loads of proteolytic enzymes. But this malarial invader rendered such vesicles futile – unable to fuse. So now the sporozoite hid securely by means of two layers of cell membranes that are impenetrable to molecules of antibody. Rounding up into a ball, the sporozoite transformed into something more sinister still.

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Outwardly the child showed no sign of the new infection that any doctor could detect. Immune memories of her older infections with the usual local strain of plasmodia remained deep within her body, readying for another fight. This new infection had become reduced to just nine sporozoites of the original batch from the mosquito. Nine minute specks of living matter on the massive scale of the liver, but the scale that counted for health or disease was measured in molecules. There

were huge molecules such as proteins, and tiny ones as simple compounds of oxygen and nitrogen atoms. The sporozoites traversing the liver cells were safe from antibody, the molecules of which were too large to diffuse through the outer membranes of cells. But they were not safe from other defenses that were well developed within dedicated killers of parasites such as macrophages, and that also resided in the multi-competent hepatocyte liver cells. The sporozoites were not sufficiently safe merely by hiding. They needed to reproduce fast enough into another form with the ability to invade an even safer type of cell: the red cell of the blood.

The nucleus of the sporozoite, so far more of a manager on the sidelines rather than a forward player, now took over completely. It divided repeatedly and exponentially, forcing the daughter nuclei outward to fill the cytoplasm that itself expanded to support these replications. The parasite cell remained a coherent whole, a sphere called a schizont, but comprising many splitting nuclei. The liver cell grew around the schizont, not merely stretching but accommodating the invader with a massive supply of nutrients. The divisions continued until about thirty thousand nuclei had been created from the original one. Then cytoplasm of the parasitic schizont gathered itself around each nucleus, and an outer membrane formed to define each new individual parasite. These new forms of plasmodia, called merozoites, remained within the original vesicle made of the membranes of their host liver cell.

Within this disguise, the cloaking vesicle or merosome, thousands of new parasites squeezed out of the now dying liver cell. The liver cell might have been dying but the usual molecular signal that would activate phagocytic cells to dispose of such a cell was inhibited by the merozoites. The vesicle pushed past the cells lining the sinusoid of the liver, past the Kupffer cells, then into the blood stream. The blood carried the vesicle rapidly through the body and the vesicle burst open to spew its cargo of invaders amongst the red cells of blood.

Antibody could not reach the developing schizonts, but more complex weapons and tactics were deployed. The weapons might work despite having been trained against the usual village strain of *Plasmodium falciparum* and of uncertain use against this newcomer. As a schizont grew, its own biochemical processing of nutrients provided by the liver cell inevitably produced by-products and wastes into the cytoplasm of the liver cell. These signs of infection alerted the nucleus of the liver cell to activate a processing of these foreign materials with molecules controlled by a group of genes known as the major histocompatibility complex. These glycoprotein molecules move from the inner cell to sit astride the outer plasma membrane of the liver cells, normally signaling to the immune system that they belong to self-cells. But when infected, the liver cells presented proteinaceous fragments of the plasmodia, as antigenic peptides, on their MHC molecules.

When these molecules became exposed to the wider body, they signaled to the immune system that precisely here in this cell is a parasite that must be killed,

whatever it takes. Away in the lymph nodes and spleen, in response to the first faint signals from the sentinel cells, other white cells of the blood, T-lymphocytes, had matured for action. These T-lymphocytes had a character known as CD8. Like the B-lymphocytes that generate antibody, these T-lymphocytes had originated as a clone from stem cells. They developed with T-cell receptors that exactly matched this new invading strain of parasite. The T-lymphocytes contained tiny vesicles within their cytoplasm and these in turn contain proteolytic enzymes that could perforate the outer membrane of an infected cell, and once inside they digested the cell.

When one of these T-lymphocytes docked onto the warning flags of the MHC molecules it oriented its internal composition so that a bunch of toxic vesicles came to the contact surface, burst out and flooded over the infected cell. The liver cell died and the developing schizont inside died with it. The T-lymphocyte closed up its outer membrane, reorganized itself and continued its search for invaders.

There were other defenders on the way. These were T-lymphocytes of the CD4 variety, helper cells that could also detect the warning flags, then orient against the infected cell and respond by releasing a pulse of a specific chemical messenger, gamma interferon. This small labile molecule penetrated into the cell where it stimulated two types of enzyme of the cell.

One enzyme catalyzed the production of nitric oxide (nitrogen monoxide). The other enzyme catalyzed the production of the superoxide of oxygen. This form of oxygen is one of the intermediates in the normal pathway of energy generation in the cell when oxygen is converted into water during a series of steps involving transfer of electrons and protons. These tiny molecules, just two atoms each, are potent and vital parts of the normal workings of any cell. But in the infected cell they combined into a more stealthily fatal oxidizing agent called peroxynitrite. This destroyed the very cell that produced it. The sudden burst of oxidation caused a combination of ordinary tissue damage, and it triggered a mechanism of natural cell suicide, called apoptosis. Death of the host cell at this stage of the plasmodial cycle killed the parasite.

The child's liver, however, was a big place to hide. Somewhere within its bulk there had remained nine sporozoites that managed to turn into small schizonts. For the hunting lymphocytes to chance upon the signals warning of infection before the merozoites were mature was a race. Chance was on the side of this swarm of invaders, now explosively replicating. Although only five of the small schizonts survived and grew to produce their battalions of merozoites, five doses of thirty thousand merozoites each could be enough to overwhelm the lymphocytes.

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Blood is thicker than water – packed with red cells, or erythrocytes. Simple cells, so dedicated to their one task of carrying oxygen deep into every organ that, as they mature from their stem cells, they each dispense with their nucleus. This is a major efficiency, but burdened with a contrary weakness. The red cells become ideal as the next home for plasmodia. Merozoites floated in the plasma, colliding with the densely packed red cells. Each merozoite measured only one micrometer across (one thousandth of a millimeter) so was easily attracted electrostatically to the ten times larger disc of a red cell.

Each merozoite packed a lot into a minute space. Pear shaped, with its nucleus occupying much of the blunt end, it was armed with a penetration apparatus poised at the sharper end. There were two organelles – the mitochondrion and the apicoplast. At the penetrating end there was a complex comprising a pair of rhoptries and ten micronemes, all bound round with three polar rings which in turn were anchored to the depth of the cell by microtubules. The remaining space was packed with sundry granules. Everything worked together for invasion and reproduction, whilst evading the child's immunity.

The penetration apparatus became activated as the merozoite stuck to the surface of a red cell. Molecules protruding from the outer membrane of the merozoite matched up with specific molecules on the red cell. The merozoite oriented to place its penetrative end against the red cell's surface and an intimately tight junction promptly formed between the two surfaces as their molecular surfaces mutually attracted. Suddenly the red cell surface convulsed and collapsed as a deepening cup. Tubes in the merozoite contracted to push the penetration apparatus deeper in. As the merozoite penetrated, it discarded its surface coat into the plasma of the blood. All was completed within thirty seconds as the cup in the red cell enveloped the whole merozoite. Finally the lip of the cup closed and coalesced to form a membranous vesicle around the merozoite.

There now sat the merozoite, within a membrane, the parasitophorous vesicle, which in turn was within the outer membrane of a red blood cell. This red cell, without a nucleus, had lost its ability to process the antigenic proteins of an invading parasite. It could not deploy distress signals crying out to any passing competent white cell: attack and digest both me and the parasite infecting me. Instead, the merozoite quickly sank below the scanning horizon of the child's immunity.

The quicker the better to avoid antibodies that would soon be circulating in blood in increasing concentration. Antibodies that would accurately detect and latch onto the particular proteins on the outer surface of merozoites as they briefly floated naked in the blood plasma. Any such coating with antibody would render a merozoite both incapable of invading a red cell and liable to phagocytosis by macrophages.

The merozoite engulfed droplets of the material making up the red cell into its digestive vacuole. The food for the merozoite's reproduction was mostly

hemoglobin, the oxygen carrying protein of blood, and this was digested with the aid of enzymes. The merozoite grew and started to divide, as a second type of schizont, to form daughter merozoites. Two days were spent going through four series of divisions, stretching the red cell to bursting with release of sixteen new merozoites into the plasma. Soon each of them in turn would be either infecting another red cell, or succumbing to antibody and macrophages. The merozoites were still few in number amongst a vast number of red cells. For a while – but all too soon the child would become aware of sickness within.

A merozoite took in much hemoglobin and had to process this to rid itself of the potentially toxic part of the molecule that contains iron. Crucial to the function of hemoglobin as the carrier of oxygen, this iron lies safely at the center of the huge molecule. But as digestion by the merozoite proceeded the iron was exposed and oxidized, stimulating the release of the superoxide form of oxygen that could kill the merozoite. Instead the merozoite commandeered a specific enzyme to deactivate the superoxide. The merozoite's digestion of hemoglobin resulted in the eventual formation of a much simpler molecule called hemozoin. This toxic material was discarded into the blood plasma when the red cell burst apart. Not simply toxic, the hemozoin suppressed the activity of immune sentinel cells as the first line of defense against plasmodia and other invaders.

The child began to feel ill: initially a vague malaise but soon followed by fever. The sequence of infection of red cells followed by replication of the merozoites followed a two day cycle. With every cycle the number of merozoites in the blood increased ten-fold and the child's fever raged when hemozoin was released. Her immune system was working as hard as possible, exhausting her reserves of energy. A cascade of interconnecting inflammatory reactions to all these parasites flooding directly into her blood plasma brought on fulminating malaria. Bizarre symptoms appeared, paradoxical sensations of shivering coldness followed each burning fever.

The red cells were not, however, an impregnable bunker. Despite their lack of nuclei they retained a trace of the capacity that normal nucleated cells, such as liver cells, have for processing the antigens of invaders and to place them as warning signals on the surface of the cell. So some of the infected red cells got a coating of specific antibody and this enabled macrophages to engulf them. Moreover, as any red cell was infected by a merozoite the invasion left molecules from the merozoite out onto the surface of the red cell. There are various types of molecule involved, such as the one called erythrocyte membrane protein. Out on the surface of the red cell, such an antigen from *Plasmodium falciparum* would signal to immune cells that this cell was infected. But this identification had to be exact; it would only work if there was a precise match with receptor molecules on the outer surface of lymphocytes and macrophages. These immune defenses had evolved to kill invaders only, if they deploy their deadly granules and secretions and tentacles too freely the whole healthy body will suffer.

For the child, her immunity struggled with this plasmodial protein coating the surface of her red cells. Had her body acquired immunity to it before or was it a slightly different form? There are sixty different genes for this protein, each of which can code for another variant. The merozoites slowly cycled through their repertoire, every slight variation another disguising cloak for the plasmodia to hide behind. If the erythrocyte membrane protein was new then several weeks would elapse before the child's immunity acquired a fully effective response to it. And so the fight clashed on with a push here, a retreat there. Ever more evasions, disguises and decoys were deployed by the plasmodia against the weapons in the multiple immunities of the child.

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Her mother had witnessed these fevers before and knew them from bitter personal experience. But now she saw something new and strange happening – her daughter seemed to be drifting away to a place she had never travelled before. The mother called her nephew to seek somebody in the village with an automobile or truck who could take them to the hospital in Kalene. Not far away, but a struggle along a gravel road now churned to clinging black mud in this season of rains. At the hospital the admissions nurse recognized yet another acute case requiring an immediate blood sample for diagnosis.

In less than an hour the laboratory technician had smeared a drop of the child's blood thinly onto a glass slide, stained it deep blue and purple and peered within the red blood cells under a microscope. Just a few, minute but distinctive, there lay plasmodia – blue, purple, and within the greyness of the red cell discs. He saw not just the ordinary stage in the blood, the merozoites. Under the highest magnification of the microscope appeared the elongated forms of the parasite, bent into crescent shapes tight within the confines of the red cells. These were sexual stages of the plasmodia, from genetically female and male cells that had developed as an offshoot of the regular cycles of asexual replication of the merozoites. The technician recognized the warning: this infection was well advanced, crisis impending. By now there could have been tens of thousands of plasmodia in every milliliter of blood, in every quarter teaspoonful.

Not just dangerous to this child but now dangerous to those around her. Once any sexual forms of the plasmodia got back into an *Anopheles* mosquito that might feed on the child, they would fuse as an egg stage, a zygote. The genetic mixing that followed would add more possibilities for variant forms of the plasmodia to evade better the immunity of people. Similarly in humans, reproduction with two sexes has a matching function of enabling a vast variety of possible immune defenses. Host and parasite engage in endless battles of camouflage, subterfuge, calibers of weapon and multiple redundancies.

The duty physician prescribed the drug often used at this hospital against this parasite. Quinine: it was one of the original anti-malarials, extracted from the bark of the cinchona tree, but remained effective for emergency treatment. A last chance treatment because the plasmodia were progressing toward their most extreme tactic. When a merozoite infected a red cell, that cell lost much of its flexibility and slipperiness that was essential to its normal ability to be squeezed by pressure from the heart along the minutely narrow passages of the capillaries.

When infected red cells stiffly jostled deep in the brain, some of them jammed there. Stuck not only like bricks, they were stuck to each other and to the inner walls of the capillary. Their stickiness derived from the same erythrocyte membrane protein that served the merozoite as a disguising cloak worn by the red cell. Clumps of infected red cells became sequestered in the child's brain, safe from the immune filtering of the spleen. These sequestered merozoites continued to replicate.

In her hospital bed the child approached the crux of her battle with *Plasmodium falciparum*. The clumps of sticky infected red cells deprived spots of her brain of sufficient oxygen. Immune white cells, clustering around these infected red cells, fired off their chemical messages and toxic granules, but in a dangerously confined space for such powerful weapons. The groups of macrophages around the sequestered red cells in the brain produced nitric oxide and the superoxide of oxygen. Again peroxynitrite was formed as a powerful killer of microbes and parasites.

But too much of this potent chemical amongst the clumps of sequestered plasmodia within the brain damaged the ability of nerves to transmit impulses. The child swung in and out of consciousness and her body heaved with the contortions of cerebral malaria. Her blood had lost much hemoglobin making her anemic, and her body tissues had become acidic. Even the quinine had the side effect of reducing the sugars available in her blood, depriving her of the energy she desperately needed.

She won. With the help of her mother's love, the hospital staff, and the quinine, she lived on. But her mother would worry for years whether her daughter's battle with this remorseless foe had left her with some diminished ability. How well would she fare when she started school?

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This story of a malaria parasite and its interaction with a person's immunity is partly true – a composite of knowledge of species of *Plasmodium* in various species of host, but rarely from humans because it is so difficult to study this disease directly in people. My account has some approximation to objective reality as it occurs in the natural world, but no better. It derives from many disparate descriptions of bits and pieces of that reality that have been made accessible by an

enormous variety of researchers, rarely in full agreement with each other, working tirelessly and with fantastic ingenuity.

Let me give an impression of a single technique of research, selected for its simple vocabulary. The precise movements of sporozoites as they invade a liver are studied because their passage from the skin to the liver cell exposes them to the effect of a vaccine. How is possible to track them? By video photography! Instead of examining a piece of liver as transparently thin razored slices in which to search with a microscope, the slicing is done by light. The researchers can thereby keep the liver alive and observe the movements of wriggling sporozoites in three dimensions.

But a sporozoite is just a translucent wisp. To render it visible the researchers will have inserted into it the genetic code for a protein that glows green when illuminated. This green fluorescent protein was first found in a species of jellyfish common in coastal waters of America, it self-illuminates small spots around the jellyfish's outer rim. More about such genetic manipulation later in the book – the upshot is that live sporozoites glow green against the dull background of the liver.

One photon of ultraviolet light is all that is needed to excite one molecule of the protein, but this type of light damages cells. Instead the protein is excited using two photons of light of a longer, safer, wavelength. But two such photons must arrive simultaneously at the protein molecule to do their work and the way to ensure that is to pack the photons together a trillion times more tightly than usual. The laser source of light delivers extremely short bursts and these in turn are focused onto the liver down through the lens of the microscope. The spectacle of a crescent shaped sporozoite gliding along the sinusoid of a liver is captured with a video camera mounted on the microscope. (See 'Sources and notes' for a simulation.)

Obviously this story of malaria is an approximation because so simplified and compressed; the full information on paper about malaria would overflow the libraries of a university. Worse than that, such information is doomed almost forever to be an approximation because the deeper researchers observe and experiment within the complexities of the molecular tricks and contortions of *Plasmodium* and the intricate maze of human immunity the less it is possible to give any single summary account.

None of this is sufficient impediment to continuing on to our technical fix. This is one fix of many now available, out of a large array of varied solutions tried in the past. Fixes including the attempt started in the 1950s to eradicate malaria by killing mosquitoes with DDT insecticide, and the invention of chloroquine as a synthetic mimic of quinine that could be taken prophylactically. Both of those fixes succumbed partially to the ability of mosquitoes and *Plasmodium* respectively, to mutate into resistant strains. Only partially: both these chemicals remain in some

limited use and alternative versions continue to be invented and deployed despite often repeated predictions of impending failure to maintain the rate of invention.

A killer of children on a global scale as *Plasmodium* deserves the attention devoted to killers and maimers like the viruses of smallpox and polio; the first truly eradicated from the Earth (except for two samples stored in USA and Russia) and the second close to eradication. Both were fought using mass vaccination. However, although some viruses and many bacteria have proved easily susceptible to vaccines, others such as the influenza virus easily slip past the protection provided by vaccines. As for protozoans, and for multicellular parasites, it seems the more complex they are the more difficult it is to devise a vaccine against them and the more difficult to ensure the vaccine is sufficiently comprehensive and effective.

So eradication of malaria will take more than a vaccine. But the modern ability to manipulate molecules combined, with the deep collective understanding of malaria, now encourages more funding and research on a vaccine. Hopefully the advantage of an effective vaccine over the insecticides to kill mosquitoes and the drugs to kill plasmodia is that the problem of acquisition of resistance will be avoided sufficiently. Furthermore, when vaccines work well they do not have to be delivered year after year, a primary shot followed by some boosters often suffice. This should be easier to deliver than repeated doses of anti-malarial drugs. These need to be taken almost continuously for full protection because of the frightening ability of plasmodia to acquire resistance to the drugs if there are any lapses in levels of the drugs.

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A doctor developing an early vaccine against malaria, W. Ripley Ballou, came down with the disease. He was shocked, despite having been exposed to the plasmodia. After all, he had been vaccinated and was eagerly anticipating exciting developments along the way to an anti-malaria vaccine sufficiently developed to be registered and put to use. He was then, 1987, working as an officer in the Walter Reed Army Institute for Research, just north of Washington DC – a laboratory with a long history of battles with malaria. One of the many dismal facts of warfare is that in many campaigns more soldiers have been killed by agents of infectious diseases than by enemy combatants. Army and naval laboratories to study and prevent these losses have made many contributions to protect their own forces, and these improvements transfer into civilian medicine.

Doctor Ballou volunteered to receive a trial anti-malarial vaccine, then be exposed to plasmodia from bites of infected mosquitoes. And so, when remaining healthy, demonstrate the vaccine worked. This was an early vaccine of a new type, less effective than he hoped, but he was cured with drugs according to the emergency plan. Volunteering yourself as a test animal, a well established tradition

in the infectious disease business, has yet to be banished in this current era of over-regulation, especially in the case of human malaria which is specific for us.

Ballou, more determined than ever from his frightening experience, went on to become one of the leaders of research that has produced the type of vaccine against malaria with the largest and widest clinical testing. Aimed specifically at infants, it has been tested widely and for many years in Africa south of the Sahara, where malaria caused by *Plasmodium falciparum* is more intense and devastating than anywhere else in the world. Originally it was named RTS,S/AS..., a cumbersome abbreviation I will explain later. For this book calling it 'sporozoite-vaccine' will suffice. This is the technical fix most appropriate to explore further with you. First, we need some history.

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A vaccine against malaria was tested about 1941. It gave some protection to birds. Yes, birds: they suffer from their own type of malaria. A colleague of mine used to astonish our students by telling them of a type of malaria in penguins at the city's zoo – the parasite is transmitted by mosquitoes between penguins and local birds roosting in a nearby wood. One of the discoveries of how malarial parasites are transmitted by mosquitoes was made by Ronald Ross working in Kolkata, India, in 1898 with a species of *Plasmodium* of sparrows. Ross found birds easier to manage than his patients.

That vaccine, although funded by the Rockefeller Foundation and developed in a malaria laboratory, was crude even by the standards of its day. The researchers ground up parts of infected mosquitoes – the thorax containing the salivary glands and their load of malarial sporozoites. Crude though it was, partially effective and only in chickens, it was the first tentative step on a journey that led, haltingly and tortuously to our current sporozoite-vaccine. The stage of the malaria parasite those researchers in India targeted was the sporozoite; hoping to halt the disease before it became established deeper in the body.

Research on malaria during the 1940s concentrated on the urgent need for a synthetic substitute for natural quinine, then scarce because of the disruptions of wartime. Chloroquine was discovered in 1934 and similar chemical drugs were found by 1945 amongst fourteen thousand compounds screened. By 1939 the powerful insecticide DDT was found amongst a ragbag of synthesized chemical oddities. It was a vast improvement on the general poison arsenic, which had been used in controlling malarious mosquitoes. This new material was a genuine insecticide, specifically and acutely poisonous for insects. So now the plasmodia could be killed in the blood by regular, prophylactic, doses of cheap and effective drugs whilst the habitats of mosquitoes were sprayed with DDT.

The eradication of malaria suddenly rose high up the political agenda and the Fourteenth World Health Assembly in 1955 adopted this as a principle. Two

years later the World Health Organisation started a campaign to eradicate malaria. The plan, however, was vague, lacking even definition of whether it was to eradicate the disease or the plasmodia. Also it lacked focus on Africa as the most severe endemic continent. The main means was by mosquito control. Implementation of the campaign over the next decade competed with the effective campaign to eradicate the virus of smallpox, also run by the WHO. Nevertheless, with massive effort and money going into eradication, who might be bold enough to investigate a vaccine against malaria?

Ruth S. Nussenzweig, working in the medical school of New York University, understood from her upbringing and medical education in Brazil that there remained a future for a vaccine. In Brazil she had learnt of the confounding complexities of a mosquito eradication campaign that had been run in part of that vast country. Moreover, support for vaccine research became easier to obtain as resistance was acquired by both plasmodia and mosquitoes to the new chemicals deployed against them by the 1950s. The US Army provided funds for a small project in her laboratory on First Avenue of the city, to test a way of vaccinating mice against a species of *Plasmodium* they naturally suffer from.

Again sporozoites were the focus, but this time with a combination of delicacy and power appropriate to such a formidable microbe. Mosquitoes bred in the lab were infected. When the infection matured the mosquitoes were pulled, apart one by one. Gently, so that their large paired salivary glands emerged from the thorax as the head was slid off, all in a drop of saline solution. The glands were disintegrated to release the sporozoites which were then bombarded with X-rays. The notorious effect of these rays on the genetic capacity of living things, as high energy photons collide with genes along the DNA molecule, was anticipated to prevent the live sporozoites from developing through their life cycle. Nussenzweig and her colleagues then delivered these irradiated sporozoites, massive numbers of them, as a vaccine. They recorded some protection against further infection in the mice, and were encouraged to extend the project to human malaria.

Ruth and Victor Nussenzweig, with an extensive series of collaborators and volunteer vaccinees, achieved high levels of immunity in people when the irradiation of the sporozoites was done whilst the plasmodia were still in the mosquitoes. Where to take this method next became an increasing problem. This was few people's idea of how to produce material for mass vaccination. Contrasting with all the talk about design of high-tech vaccines as the modern improvement on guesswork, trial and much error, this was worryingly low-tech and thus poor way to inspire commercial investment. Design was hampered not only by lack of knowledge of the precise immune mechanisms working against sporozoites under conditions of natural infection. There were no other methods to culture or replicate the sporozoites and their antigenic proteins that surely were inducing the immunity.

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Help for vaccinologists was soon to hand from a completely unexpected direction. In 1984 news of the invention of a method to synthesize insulin spread fast through medical schools and clinics. Insulin, for the treatment and management of diabetes, was then being harvested from the pancreas glands of cattle and pigs cut out at the slaughterhouse. Demand was expanding steadily and supplies were predicted to be inadequate. A route had been carved out from James Watson's and Francis Crick's discovery of the structure and function of DNA through to the invention of synthetic insulin by Herb Boyer and Sidney Cohen. The route following a direct line of basic research to applied research to pure market driven invention. The inventors described in their patent how to manufacture individual proteins by manipulating DNA so that the protein could be mass-produced in ordinary cultures of bacteria or yeast cells. That is another story, not only of a superbly successful technical fix, but of a true revolution in science and the birth of the industry of biotechnology. Chapter 2 tells it.

For the Nussenzweigs, for Ballou, and for everybody else groping in the dark maze of the immunology and vaccinology of malaria a door swung open onto bright new vistas. By the early 1980s the techniques for working out the sequence of the sub-units of DNA that make up the gene carrying the code for a specific protein were applied to malaria. Fidel Zavala, with the Nussenzweigs, described a protein that forms a distinct coating all over the outer surface of a sporozoite. If sporozoites were exposed in the laboratory to solutions of antibodies previously raised against sporozoites, the antibody bound so tightly to the coat that it neutralized the sporozoite. Stopped it from functioning, killed it. Zavala named the coat as circumsporozoite protein. Surely this was the one to focus on – already it was well known that whole sporozoites could constitute a vaccine. Highly specific antibodies could react with it and so kill the sporozoites.

John B. Dame and colleagues at the National Institute of Allergy and Infectious Diseases in Bethesda, Maryland, then spelt out the entire sequence coding for the gene of the circumsporozoite protein. Finally, in this rush of conversion of malaria vaccinology from grinding up parasites to genetic engineering, James F. Young published a paper describing how to produce a synthetic version of circumsporozoite protein. He was with a large team spread across the Walter Reed laboratories, the Naval Medical Research Institute in Maryland, and the pharmaceutical company then known as Smith Kline & French Laboratories.

By 1985 there were two routes open to producing a synthetic mimic of those parts of the circumsporozoite protein known to be most active at inducing a strong immune response. One route, a chemist's style, was to assemble the immunologically relevant sub-units of the protein, in the form of amino acids, to make what are called synthetic peptides. The other route, more biological in style, was to trick microorganisms into producing the required segment of the

circumsporozoite protein. The result is called a recombinant protein, named after the subtle action of an enzyme in the manipulation of the DNA (the root word recombine here has a different meaning from its use in the genetics of sexual reproduction). The team in New York took the synthetic peptide route whilst the Smith Kline & French and Walter Reed workers formed a formal collaboration for the recombinant protein approach.

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The vaccine of our story, the sporozoite-vaccine, is formulated with a recombinant protein. It is complex as you might guess from its gnomic abbreviation and to explain it I will need to divert into a sub-plot. This is a distinct technical fix story in itself because its disease causing organism, a virus, is amenable to the attentions of vaccinologists.

Within RTS,S/AS... the **R** stands for repeat. That is: repeats of a row of identical sequence codes along about one third of the string of sequences for amino acids as represented in a line diagram of the protein, or its primary structure. These were selected for their ability to induce good antibody. They also induce good cellular immune responses, hence the **T** for T-lymphocyte. Neither **S** is for sporozoite however, but for what is known to vaccinologists as the S-antigen. This is from the surface coat of the virus that causes the liver disease hepatitis B. The second **S** in the abbreviation is more of the same material, but coupled separately as a major proportion to the rest of the construction. **AS** stands for adjuvant system – more of that later, and the abbreviation usually comes with a serial code for a version of the vaccine. So the **RTS** is a hybrid synthetic protein derived from genetic information of two disparate parasitic organisms, a protozoan and a virus.

Vaccinologists sometimes call such combination a chimera. They could be tempting fate: a fabulous beast made up of parts from various animals, or a wild and unrealistic dream? The S-antigen has the inherent property of spontaneously folding up into spheres, about the size and shape of a virus. Without a genetic core it cannot replicate but it appears to human immunity like an invading agent of infection. Immune sentinel cells are alerted by it.

Where and how did a vaccine against malaria get into bed with an imitation of a hepatitis virus? This story starts with Baruch S. Blumberg, doing medical research from 1964 at what was then called the Institute for Cancer Research, Philadelphia. Rather than tackle cancer head on Blumberg continued with the highly individual approach he had cultivated since being a medical student and then consolidated whilst first doing academic research in the University of Oxford. He sought markers of diseases to be found in blood, in the innumerable variations of groups and immune characters of blood from different people and countries.

One of Blumberg's colleagues found a most unusual protein in a sample of blood from an Australian. They nicknamed it Australia antigen. There it remained, a half-remembered oddity in the freezer. For a while the team thought they were on the track of another marker for leukemia, but instead found more Australia antigen sufficiently frequently for the data to resolve into vague patterns of the sort that fascinate epidemiologists. Could this be a marker for an infectious disease? The patterns hinted at a virus; confirmed by finding some under an electron microscope. Further tests revealed it was identical with the virus causing hepatitis B. Australia antigen soon was revealed as more than a marker for the virus, it was the outer coat of protein normally wrapping around the core genetic material of the virus. This protein was found, by itself, floating in prodigious quantities within the blood of people suffering long-term infection with the virus.

The team's collective imagination stirred and the idea of trying to produce a vaccine crystallized. Their technical problem was how to produce this S-antigen as they now called it, in a form suitable for manufacturing a vaccine. Because it formed naturally into discrete spheres, they found it simple to separate the protein from the fluid portion of blood, the plasma, by spinning plasma in a centrifuge. Plasma was obtained from suitable donors at blood transfusion clinics, where the clinicians needed to detect this virus to improve infection control. Early experiments proved the S-antigen produced strong antibody responses that seemed to be protective; the antibody could kill invading virus.

Hepatitis B was then becoming rife amongst gay men in New York City and the first clinical trial was held there amongst those men who volunteered for a shot of either a placebo of saline solution or a shot of what was becoming known as the plasma vaccine. The trial was such a success that the job of manufacturing the S-antigen into a form safe and efficacious for registration and sale needed to be handed over to commercial vaccine makers. At the Merck & Company laboratories in New Jersey the formidable vaccinologist Maurice R. Hilleman rolled up his sleeves and took charge of the project in military fashion he had learnt whilst at the Walter Reed labs. His only source of the S-antigen was plasma from blood donors. People infected chronically with hepatitis B virus, and maybe other microbes. Those were still the innocent days before knowledge of human immunodeficiency virus, and of prions as the cause of brain disintegration, was part of routine medical business. Hilleman's stringently severe purification and sterilization process would have killed the agent of AIDS, but prion infections are almost impossible to avoid in such a setting.

The revolutionary ability to synthesize proteins in bulk, using knowledge of their genetic coding along the DNA molecule was a rare gift of good timing. The recombinant protein version of hepatitis B vaccine became available just a few years after recombinant insulin. That vaccine was the first synthetically manufactured vaccine registered for humans. One of the firms that manufactured it, GlaxoSmithKline, was an obvious partner to produce recombinant proteins as

experimental malaria vaccines in sufficient quantity and quality for large clinical trials.

Joe Cohen worked for them and was quick to see the potential of using the self-enveloping S-antigen as a carrier for the proteins of a malaria vaccine, as hopefully a stimulant to immunity, and at the least a collateral vaccine providing protection against the virus of hepatitis B. The entire hybrid protein, the RTS, is grown in ordinary yeast cells as a single recombinant protein, whilst the same cells churn out a larger proportion of the plain S-antigen. From the first recombinant circumsporozoite protein of James Young and colleagues to the proof-of-concept trials with Joe Cohen's vaccine took 12 years. Finally, about 1996 full clinical trials could start.

This sporozoite-vaccine was a very different proposition compared to the recombinant hepatitis vaccine. Early versions of recombinant proteins to mimic parts of the circumsporozoite protein induced modest immune responses in the form of antibodies and variable, fickle activity of T-lymphocytes. The efficacy as vaccines, measured in small trials, were in the zero to twenty percent range. What effect they did offer was difficult to explain. How much was antibody able to neutralize the progress of sporozoites on their journey through the skin, blood and liver sinusoids before they penetrated liver cells? When in liver cells how vulnerable was the schizont stage to T-lymphocytes and macrophages sensitized to the circumsporozoite protein?

Such questions were asked of what happens deep within the body of a human – the only place where *Plasmodium falciparum* lives the full mammalian segment of its life cycle and the only place where this type of vaccine may stop it. But if all you have, as a window onto what is happening amongst that intricate immunity, is a small tube of blood drawn from a vein, then you face severe technical problems. There is far too little that can be done with the serum, lymphocytes and macrophages isolated from the whole body to answer such questions. Human immunity works within human bodies. The ability to replicate and experiment with parts of it in plastic dishes is as limited as studying the behavior of wolves caged in a zoo. The options for rational design are bleakly sparse: try it and see remains the main way forward. At the very least make sure the vaccine is safe; hence the need for the vast body of in-house expertise that follows along the route starting with the vaccine against hepatitis B.

Adjuvants are there to ease progress, literally assistants, stimulators of more powerful action. An adjuvant for a vaccine will both carry the antigens and create conditions to induce stronger immunity than if the antigens are delivered alone. For the current version of sporozoite-vaccine there is an oily component that encapsulates the protein particles and slowly releases the active component of the vaccine. There is a material that mimics the outer cell wall of an invading bacterium to stimulate generally a frenzy of warning signals from the sentinel cells of immunity. Another component, derived from the soap-bark tree, helps to present

the vaccine components in a physical form more potently presentable to the antigen-processing cells. Precisely how the adjuvant mixture is formulated is routinely a proprietary secret and there is little evidence of deep understanding of how such adjuvants work. An art and craft more than a rational technique surrounds the precision of the hybrid protein at the heart of this vaccine; a jewel wrapped in newspaper.

Progress, nevertheless, is made. An international consortium of funders of malaria vaccines in 2004 agreed strategic goals. By 2015: 'To develop and license a first-generation malaria vaccine that has a protective efficacy of more than 50% against severe disease and death and lasts longer than one year.'. For 2025 the goal was stated as '. . . for 80% efficacy against clinical disease and lasting longer than four years.'.

In July 2015 the vaccine was registered by the European Medicines Agency for use. Registration was based on field trials at eleven sites spread between Burkina Faso, Gabon, Ghana, Kenya, Tanzania, Malawi and Mozambique between 2009 to 2014. Fifteen thousand infants and youngsters were given three initial doses of vaccine followed in most cases by a booster at eighteen months. At best the vaccine gave thirty six percent protection, or to put it another way an average of 1,774 cases of clinical malaria were prevented for every 1,000 children vaccinated.

GlaxoSmithKline gave the trademark Mosquirix to the vaccine and the World Health Organisation plans to coordinate national deployments of the vaccine for routine use, aiming to start in 2017. Funding has come so far from philanthropists – notably the Bill and Melinda Gates Foundation, and from international aid agencies, private research charities, military departments, funders of university research, and cross-subsidy within pharmaceutical companies. Most importantly for the long term future of such a vaccine is support from both the ordinary people and the governments in the endemic countries. Their ability to create the wealth to pay for a vaccine will be needed to maintain vaccine use sufficiently to defeat *Plasmodium falciparum*.

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It is easy to find quotes from influential people about their plans and hopes to eradicate malaria. At best this is the management technique of positive thinking, at worst it is ignorance of the heavy burden of implications that the word eradicate carries. Since the international consensus strategy is for eighty percent reduction of clinical disease, amongst those people who receive the vaccine, there will remain a proportion of people continually re-infected with threatening numbers of plasmodia in the years ahead. Those people will form a reservoir of infection to other people. The size of this reservoir might naturally decline small enough that the transmission cycle is broken, but this depends on many dynamic factors.

The understated sub-text of such plans and reviews of research contains an acceptance that this technical fix, this vaccine, will contribute to reducing the burden of the disease called falciparum-malaria suffered by people in the endemic countries. Of course, it is expected that the vaccine will be improved, there are many other types that can be incorporated. Moreover, the disease usually referred to is that variety caused by *Plasmodium falciparum*; the more widely spread and less virulent malarias caused by *Plasmodium vivax* and the other two species infecting humans are often not mentioned.

The eradication of the virus of smallpox from Earth was feasible for the heroes of that campaign because the virus infects only humans, it is transmitted only by close contact, the killed whole virus delivered as a simple vaccine gave full protection with one shot, and that shot could be delivered by something as simple as a forked needle. Natural survival from a mild infection of smallpox gives life-long immunity: the vaccine mimicked that. The logistics of vaccinating the majority of humanity were colossal, but eased by the simple biology of the disease that affected rich and poor people alike throughout the world. The vaccine to protect against the virus of hepatitis B, given the separately available and revolutionary technology of recombinant protein production, went from concept to licensing and widespread sale in about ten years. The difference from a malaria vaccine is in the biology at the heart of the matter. The peculiarly amenable nature of that hepatitis virus, with its detached S-antigen floating in human blood, could not have been easier to isolate as the original plasma vaccine.

More fundamental than strategies for true eradication of diseases is the social and economic context of malaria. Poverty makes malaria worse and in consequence malaria makes poverty worse. This circular relationship has been argued over since the early days of the League of Nations planned anti-malaria campaigns and continues with studies of the economics of the disease, without conclusion about which comes first. As with the conundrum of which came first: the chicken or the egg, an evolutionary perspective helps because eggs evolved many millions of years before chickens.

Parasites like plasmodia evolved in close synchrony with the evolution of blood feeding insects. One of the hosts of blood feeding insects and early plasmodia were monkeys and apes living in warm wet forests. Their living conditions were not poor, since poverty has meaning only in the cultures of human civilization. But these primate animals got plasmodia into their skin many times each year from biting mosquitoes. The plasmodia thrived where they could pass from animal to animal frequently enough to both flourish in non-immune young livers and blood. All it took for the cycle to keep rolling was sufficient stagnant pools of water for mosquito breeding and easy access to more animals exposed at night as they slept in the tree canopy. The mosquitoes thrived where it was green, humid and warm for sufficient months of the year. Those primates included our evolutionary ancestors.

The combination of fundamental biological conditions of the focus of *Plasmodium*, *Anopheles* and *Homo*, is known as a nidus, a nest. As an ecologically defined but physical entity, this nidus probably developed at the same time that humans developed as a distinct species – two million years ago. These ecological conditions were little different from the conditions of that variety of human poverty still now found in slums and remote villages in the humid tropics. One dollar per day poverty, scratching at the earth for a living, sheltering in flimsy decrepit houses. Such harsh poverty initially increases risk of malaria in the life of any human individual reared in an endemic area. Later, malaria worsens that poverty by weakening vitality: a trap of strong positive feedback.

How to escape from this trap? The United Nations Sustainable Development Summit, September 2015, established a fresh and comprehensive set of goals, the first of which was poverty reduction. An ambitious target of eradicating extreme poverty (less than US$1.25 per day level) by 2030 was announced. To many people this will seem hopelessly optimistic, particularly when seen from the perspective of relative poverty. That is, the type of poverty expressed as: 'Most have little, a few have lots.' This highly skewed distribution of wealth amongst people was described long ago by the economist Alfredo Pareto in the first formulation of what is now often known as the 80:20 rule. This type of non-linear distribution, described by power laws, has been found everywhere and is common in living systems. In his home of Italy eighty percent of wealth was in the hands of twenty percent of the people. The ratio in some countries is now 90:10.

But the development goal concerns the deepest poverty, not the greatest riches. Progress toward alleviating poverty, during the time covered by this story, from the 1950s onwards, has been so steady in so many countries, that the increase in human wellbeing is already contributing to specific goals such as reduction in malaria. People can afford to build houses better proofed against mosquitoes, they can install piped water, they can buy bed-nets and anti-malarial drugs.

Most of humanity used to be at threat from malaria caused by one or more species of *Plasmodium*. The most widespread, *Plasmodium vivax*, was the agent of malaria from Sweden to Argentina, from Canada to Australia via Madagascar. I knew a lecturer in tropical diseases who took his students to a site 25 miles from the center of London where he showed them the species of mosquito that used to be involved in *Plasmodium vivax* malaria there; the ague it was called in William Shakespeare's time. Now this type of malaria is rare in many such countries because of a sequence of agricultural intensification, industrialization, better housing, taxes to pay for public health control of mosquitoes, and use of insecticides and prophylactic drugs.

The discrepancy between what can be achieved in reduction of human suffering from malaria and the hopes and expectations placed upon singular technical fixes like drugs, insecticides, drainage, bed-nets and a vaccine derives from a category error. Any single technical fix can perturb the system. An

integrated package of fixes can, through its synergies, perturb the system more strongly. The long term results of such perturbations are almost impossible to predict because the dynamics of the system operate at the same category of complexity as the weather. The system contains fundamental phenomena and laws of biology currently without explanation of their workings. What can be achieved in a laboratory or a drug factory is at a level that usually seems miraculous when viewed in isolation. For any such miracle to work outside the laboratory, in a world where the people who need the cure most are those least able to buy the cure, is a problem in another realm altogether. Sometimes there are technical fixes that transcend these levels, a category leap, like another smallpox vaccine or penicillin. What has so far kept the technical fix of a malaria vaccine from a transcendental role is the dreadful and beguiling biological contrariness of a tiny single celled invader of the blood.

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