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Hepatitis B vaccine

'*We did not have to tell the research review committee what we were going to find before we actually found it.*'

(Baruch S. Blumberg)

In New York City during the late 1970s, a physician enrolled a thousand volunteers in a trial of a vaccine against hepatitis disease. The volunteers knew the vaccine was experimental but not if they were receiving doses of the real thing, or of a placebo. Neither did the physicians, who took the samples and recorded the outcomes in the volunteers, know which was which. But everybody knew that this disease was nasty, increasingly common, spread by contagion, and led to chronic jaundice or death from liver cancer.

If this was a bold gesture by the volunteers, it was nerve racking for physician Wolf Szmuness and his vaccination team from the New York Blood Center. Nobody had ever tried a vaccine made in this way before. The manufacturers extracted the material for their vaccine from the blood of people suffering chronic infection with the virus of hepatitis! This was reminiscent of the earliest immunisations against smallpox. These used the virus of human smallpox to stimulate immunity in humans against the same virus – a tricky precedent indeed. Eventually smallpox was eradicated globally using true vaccination against smallpox with the virus of cowpox. But when the new vaccination was first used people were alarmed and the more fearful ones started anti-vaccination campaigns. Cartoonists fanned the fear with pictures of patients staggering away from their sawbones doctors with cow horns sprouting from their arms. Despite such folk-memory fears the volunteers for Szmuness's vaccine were prepared to endure three shots over six months. To the relief of both them and the medical team the vaccine gave good protection with very few adverse effects.

Manufacture of this first experimental vaccine against hepatitis B was by Merck and Company at their factory in Philadelphia. There, Maurice Hilleman had directed the extraction of the key material from blood and its purification step-by-step to produce a single pure protein. At this level he could deploy all the mature technology of vaccine manufacture, with advanced apparatus to extract the protein from blood serum followed by serial chemical treatments to kill any contaminating microbes. However, this vaccine was invented in a public medical research laboratory. At the Institute for Cancer Research, Baruch Blumberg had been preoccupied with genetic studies on different types of protein in blood as possible markers for susceptibility to diseases. Blumberg's links to cancer were tenuous. He had no idea then that his searches would eventually meet his employer's main remit, because reducing infection with the virus of serum hepatitis reduced that major cause of liver cancer. What was lurking in Blumberg's samples of blood that led him to a new vaccine?

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In the natural world of living organisms there is endless variation: a reliable grist for the mill. Many researchers have developed techniques to harvest data on variation in form and function within species. Baruch S. Blumberg, as a medical doctor and researcher was one of them. After graduating from Columbia University College of Physicians and Surgeons he researched in New York as an intern on arthritic diseases. His boss arranged collaborations with leading workers on arthritis at the University of Oxford. There, in the Department of Biochemistry, Blumberg met Anthony Allison who was also working on variations of blood in relation to rheumatism. Allison already had a major reputation in the field of blood proteins from a series of papers in the 1950s. These dealt with sickle cell disease. This peculiar problem is caused by an unusual form of the red protein called haemoglobin that transports oxygen around the body. This form, a polymorphism in the jargon, persists genetically in many groups of people who historically were exposed to infection with the malaria parasite. The polymorphic form partially protects them, so a test for it reveals resistance to a specific disease.

Allison's enthusiasm inspired Blumberg to expand into evolutionary genetics. Not that he needed much encouragement, having researched as a medical student at the hospital of a bauxite mine in Surinam, or at that time Dutch Guiana. There the differences between people of widely different origins, all with varied responses to tropical diseases, fascinated Blumberg. For him this topic could be not only be a rewarding route to understanding evolutionary genetics, also it could lead to ways of diagnosing diseases in general. At the very least, it would provide all manner of explorations and collaborations at which Blumberg was so adept.

Researchers in the early 1900s had urgently studied polymorphisms of blood proteins after they discovered the importance of blood typing for successful transfusions. During the world war of 1914-18 blood transfusion was used on a massive scale, but then the technique became routine and such research declined. A few people, however, exploited these polymorphisms to study how susceptibility to diseases (or resistance, to look at it conversely) in different peoples varies with different blood groups. At the laboratory in Oxford, Allison and Blumberg compared blood proteins by recording how far they moved through a layer of starch gel on a glass plate in response to an electric current. In the early 1950s this was the latest technique but by 1958 the immunologist Örjan Ouchterlony, at the University of Gothenburg, invented a transformative method based on immunological characteristics of proteins. Allison and collaborators in Oxford rapidly adapted this method for its ability to find better facts. They were unaware that the immunological basis of this test hid a brilliant prize.

If a foreign substance, protein from bacterial contamination for example, is exposed to an animal, then the immune system of the animal recognises it as foreign and potentially dangerous. The animal's immune system will produce antibodies against the protein and these usually are sufficient to kill the invading bacteria. The immune system recognises molecules characteristic of the foreign contamination and these molecules become defined in that context as antigens. That is, they generate production of antibody. The genius of Ouchterlony's invention was to allow the potential antigen and antibody to diffuse toward each other independently from small wells punched into a slab of gel formed from agar. Double immuno-diffusion he called it. Only where the concentrations are optimal, and if there is the specific antigen or antibody present, a thin white arc of combined and precipitated proteins reveals itself. The simplicity of this test seems ludicrous by modern standards, nevertheless highly informative.

Blumberg compared blood proteins in Britain, mainly around Oxford. For comparison, he sought a specially distinct European population. The opportunity came Blumberg's way to visit Spain in 1956. There the Basque people have a unique language pointing to a genetic background separate from most Europeans. The Basques also commonly have the polymorphism for the Rhesus negative blood group, which is a potential cause of complications during pregnancy. Blumberg's results were modest, '. . . another brick in the edifice of data on gene distributions . . .' as he expressed the matter.

Soon Blumberg needed a secure job and the obvious option was work on arthritic diseases. He and his wife returned home for a post with the National Institutes of Health in Bethesda, Maryland. Uninspired by the biochemistry of proteins in limb joints, Blumberg instead exploited the academic freedom that was encouraged at NIH to continue his work on blood polymorphisms. This research on the patterns of disease would have been classed as epidemiology, a branch of science then treated with disdain by many scientists, who regarded it as merely descriptive, lacking an experimental base of its own, and worse of all reliant on statistics to extract any sense from the stores of varying observations. Some researchers still feel the same way – the mathematical analyses of epidemiology become ever more abstruse.

Blumberg and Allison again made the nucleus of an international team working in Alaska on protein polymorphisms in the blood of Inuit and Athabascan peoples. Blumberg asked himself and his readers what he had accomplished with all this fact finding. He answered frankly by writing of adding to the store of knowledge on many polymorphic traits, of making a few feeble attempts at determining disease associations, and even that their main goal could be called stamp collecting. He was too hard on himself, and that comparison is uninformative. Better is fishing trip: an activity intrinsically satisfying but with the nominal purpose of catching an object that may be edible, requiring good technique and knowledge of the surroundings, applied with considerable patience in order to maximise chance in your favour. How long would they need to spend fishing for a new genetic marker was their main uncertainty. Blumberg repeatedly described his early research being in that style: 'It was a form of inductive science – that is, collecting data within a general conceptual framework, but without specific hypotheses; the hypotheses would be formulated after the data had been collected.' Plain old fashioned fact finding always did have a long future ahead of it. The number of facts out there in the natural world is infinite compared with the number we will ever find.

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Soon Blumberg and colleagues expanded their use of Ouchterlony's test to patients in haematology clinics who needed frequent transfusions. These patients would probably develop antibodies to unusual variants of blood proteins, so the researchers used the new test to compare them with different populations. Soon enough Blumberg and team found an interesting arc of precipitated protein on their gel plates. They named the protein *Ag*. Studies of the genetic background and disease patterns amongst the patients revealed *Ag* associated with diabetes and possibly cardiovascular disease.

After seven years work for NIH, Blumberg decided to move to the Institute for Cancer Research, in Philadelphia. He was seeking even more autonomy as a researcher and particularly the freedom to operate simultaneously in several disciplines: immunology, genetics, epidemiology, anthropology. The photographs in Blumberg's autobiography show a youthfully forceful man. He was happily at home in

Alaska, living in tents pitched on the summer tundra, through to working with pastoralists in the scrublands of western Africa, and on to mobile public-health clinics amongst villagers deep in the tropical forests of Suriname.

The ICR encouraged study of scientific problems without immediate relevance to controlling cancer. As Blumberg put it much later: 'I have always felt that this was one of the reasons we were able to pursue our work so effectively. We did not have to tell the research review committee what we were going to find before we actually found it.' A generous grant, for seven years in the first instance, encouraged them to continue work on blood polymorphisms. Harvey Alter, a physician with NIH's Blood Bank, was seconded to Blumberg's laboratory in Philadelphia to study the sera of transfusion patients. One day he found a reaction in the Ouchertlony plates unseen by anybody before. Formed in the slab of gel, between the wells of antigen and antibody, was an unusually broad arc of reaction. When stained it took up a colour different from the *Ag* material Alter had expected. Knowing this was something special he rapidly tested more samples and found a vague pattern of distribution, a possible clue to a link with disease. Although this new antigen occurred less frequently than *Ag* it was commonest in the sera of people from South East Asia and Australia. The team called it the Australia antigen; they wanted an open mind on this, without committing the material to any specific role.

By 1963, the team was testing sera from Americans with haemophilia who needed multiple blood transfusions. (Serum is the clear yellowish liquid that separates from whole blood that has formed a clot of red cells in a glass tube.) They compared sera from these people with sera from people with widely contrasting genetic backgrounds. Two of the haemophiliac sera reacted with serum from a single Australian patient. The team made some preliminary characterisations of this protein, admitting they were not fully confident they could properly call it an antigen. They developed methods to concentrate and purify the material and soon found this Australia antigen in 38 samples of a bank of 1704 sera representing 8 out of 18 populations of people throughout the world. Could this be the meaningful genetic marker of disease they had been searching for so long?

Spurred on by this strong hint of a marker, the team set about testing 659 sera from patients with a range of 16 diseases. A wide range maybe, but as it happened without any diseases caused by microbes. Eleven percent of the sera from people with leukaemia reacted with the antigen and all of those people had received multiple transfusions. At last, the team could tackle their vague ideas with specific questions. They formulated three hypotheses to test. That people with Australia antigen have an increased susceptibility to leukaemia; or that the antigen is a manifestation of the process of leukaemia as a disease; or that the antigen is related to a virus that had been suggested as a cause of leukaemia.

Ideas about the possible genetic relationships of Australia antigen most fascinated the team. They offered hypotheses that a proposed gene called *Au* would exist in two forms, or alleles. One form would code for the production of Australia antigen, the other would not. The researchers would find the antigen in persons who inherited from each parent only the same form, the form coding for Australia antigen. Individuals inheriting only one or none of the forms coding for Australia antigen would have none of it in their blood. This is about the simplest hypothesis they could have proposed using the original theory of genetics developed by Gregor Mendel. The team failed to find the expected precise sorting of the genes

that coded for the production of Australia antigen. Blumberg reluctantly concluded that the studies failed to impress his colleagues, and doing more of them was unlikely to change their minds.

Nevertheless, the team optimistically generated alternative hypotheses and searched the world for more samples. Scraps of evidence arose from the accumulating mass of data that could have hinted to them that a microbe in the transfused blood was related to the antigen, but the clues seem to have been swamped by the sheer mass of data. As scientists with backgrounds in rheumatology, biochemistry, immunology and other fields, all working in a cancer research institute, they were poorly placed to think imaginatively about microbes and infectious diseases, to think as epidemiologists.

They did know confidently that sera containing the antigen were rare in samples typical of the American populace but that the antigen was more commonly found in samples of people with particular diseases. Leukaemia was one example. Already doctors knew this disease to be more prevalent in persons also with Down's syndrome. So the team sought out blood samples from such people by negotiation with medical staff and parents of children with Down's in Philadelphia. Their hypothesis predicted that sera from these people would show a greater number of positives for the Australia antigen than sera in a sample from people without this link to leukaemia. The difference would be statistically significant. Much to their excited surprise, this is what they found.

By that time beginnings of the move toward caring for people with Down's in smaller groups and with conditions of improved hygiene was becoming effective. The team found the antigen in sera they collected from people in the new accommodations less commonly than in sera they collected from people in the older less hygienic institutions. Thus, one of their original hypotheses involving an infectious agent, probably a virus, came to mind. An idea pushing at a door through to the totally unknown.

A physician in the team, Alton Sutnick, had a patient who suddenly became positive after having tested negative for Australia antigen in his blood. The team's hospital unit admitted the boy for tests of liver function because the liver is a major source of proteins that circulate in the blood. Sutnick found characteristics of the blood indicating inflammation of the liver, or hepatitis. Doctors knew the most likely cause of hepatitis in a child was a microbe. Promptly the team submitted a paper to publicize their discovery; they must have become suddenly aware of the background of publications on serum hepatitis, alternatively known as transfusion hepatitis. Their euphoria with a discovery to announce disintegrated into dismay at the response from the referees and editor of *Annals of Internal Medicine*. Rejection. Nevertheless, by good luck and the advantage Blumberg had learnt as a doctoral student of prompt and frequent publication, already they had a preliminary report of their discovery in the same journal.

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Serendipity is the word Blumberg used to describe the discovery of Australia antigen. A talent for making fortunate discoveries by accident, as a dictionary will define it. Or a fancy word for luck as many people use it. Passive luck: not the same thing at all. Neither serendipity nor luck is something to incorporate in a grant application. As Blumberg expressed it: 'There is a school of thought in research funding which seeks to identify projects that have a high likelihood of hitting "pay dirt", either theoretically or applied. This is an approach that is hard to dispute – except by the argument that it works against the discovery of

totally new ideas, ideas that cannot be anticipated on the basis of current knowledge.' People write books and long lists of examples about serendipity in invention, but the idea is always dragged down by association with the dumb luck of the prize winner in a lottery who knows exactly what they want and ignores the enormous odds against a win. Luck is not supposed to be the business of serious scientists.

How do you develop a talent for making fortunate discoveries by accident? Start by searching for them (as in the original French verb *cercher* before this simple action was burdened with the *re-*). Persist in searching with your mind open to all possibilities, to the unexpected, to the outlier result that is so easily dismissed during statistical tidying of the raw data. Mostly all you will find is plain facts; expected but some interesting. Soon enough, in a suitably fresh area, you will come across things unexpected that nobody else has ever found. Usually small things, that once explained make a good paper for publication. Or, rarely, a fact like Australia antigen that seemed so insignificant when Harvey Alter first found it.

Can it be that cultivating serendipity is what researchers are really paid for? Researchers are reluctant to admit to this: it sounds too light-hearted. Scientists who go on to write as philosophers can be freer with their insights. As David Bohm and David Peat, in a book on scientific creativity explained: 'In other words, the creative person does not strictly know what he or she is looking for. The whole activity, therefore, is not regarded as a problem that must be solved but simply as play itself.' If simple luck is sought in this story, it lies within the event of the first antibody against the antigen that Blumberg's team found being the one that protected people against infection.

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Hepatitis emerged into medical knowledge from a shipyard in Bremen, Germany. In 1883 this was the scene of an outbreak of jaundice amongst the workers. Recently they had received compulsory vaccination to protect them from smallpox, but many of them developed jaundice and inflammation of their liver in the following weeks and months. Doctors suspected use of human serum to dilute the vaccine was causing these symptoms of disease, but their thoughts were based on association and coincidence. Nobody knew of any precise mechanism to connect this defence against one disease with emergence of another.

Over many years a knowledge built up to associate a particular form of hepatitis with the use of human serum. This continued to be used to dilute vaccines or to provide temporary protection from microbes if the serum was from a person recovered from a specific infectious disease. This serum hepatitis was contrasted with more common infectious hepatitis, which is very similar clinically but has a shorter incubation period and duration of acute illness. Into the 1940s the cause of serum hepatitis remained unknown. Was it caused by a microbe in the serum, even one of those mysterious viruses recently discovered, or could there be a toxin in the serum?

During the world war of 1939-45 vaccinations against the virus disease yellow fever grew. Despite the natural area for yellow fever – tropical regions of South America and Africa – being remote from the theatres of that war, military planners feared the virus as a potential biological weapon. Human serum was then used in manufacture of the vaccine. Returning soldiers reported sick with serum hepatitis, thus a large corps of doctors in America treated so many cases of serum hepatitis that they started to work out

the relationship between infectious hepatitis and serum hepatitis. Whatever the agent causing serum hepatitis was, they knew its route of transmission to be from the blood or serum of infected people through some form of injection done by doctors, or by direct contaminative exposure to other people.

So far, some progress – then research declined to a plod. One of the diseases was associated with poor hygiene and ineffective public health. The other was also contagious, but associated with medical interventions, an iatrogenic problem and another embarrassment to the profession. However, researchers continued to investigate the protective effects of serum from recovered patients, or gamma-globulin therapy as it is known. Saul Krugman was a specialist with this therapy. As a professor of paediatrics at New York University School of Medicine his main concern was the control of infections such as polio, measles and hepatitis. Krugman was born in New York to parents recently arrived from Russia. He had studied at the Medical College of Virginia, then at New York University but in 1941 he joined the U.S. Army Air Corp and saw long service as a flight surgeon in the South Pacific.

In the 1950s and 60s many disabled children were cared for in large institutions and Krugman spent much time working at the Willowbrook State School, of New York State. The difficulty of maintaining good hygiene in such places allowed rife infectious hepatitis. Krugman and colleagues used their access to Willowbrook's children to study long-term patterns of hepatitis. They teased apart the confused patterns of disease to propose separate causes, as two viruses, A and B, with the serum hepatitis caused by the B virus. When a top American medical journal published this work in 1967, the article was accompanied by a congratulatory editorial explaining that: 'Everything we know about the properties of the virus, the pathogenesis of the disease and the possibility of prevention by γ-globulin has been learned from carefully controlled studies on human beings. These recent studies by Krugman et al represent an important contribution to our knowledge of hepatitis that would have been impossible without the judicious use of human beings in carefully controlled experimental studies.'

Judicious use? None of these children, these patients handed over by their parents to a State care school because they had Down's syndrome, volunteered themselves. The situation at Willowbrook, and similar institutions, became a focus of intense debate on medical ethics and is now a classical case-study that rightly continues to stir up intense emotion. At least the furore soon contributed to a substantial change in attitudes and practice. Despite such a troubled start, at least the importance of hepatitis type B was becoming recognized, helped by a clear name and clinical distinction from hepatitis type A.

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One year after Blumberg's preliminary report on Australia antigen, a virologist at the New York Blood Center published a similar paper that would eventually become as highly regarded and often cited as the first. He was Alfred Prince and already was collaborating with Blumberg and Krugman. The Blood Center urgently needed a method for screening blood in order to remove batches that could cause serum hepatitis. Prince's paper was a description of a diagnostic test for contaminated blood likely to cause hepatitis. The test used the Ouchterlony plates for what Prince called the serum hepatitis antigen, but he went significantly further than technique. He declared it likely that measurement of what he called serum hepatitis antigen could be a test for a possible virus causing serum hepatitis.

Prince was being cautious, despite having firm opinions on the matter. He had worked on hepatitis as early as 1964 during service with the US Army in South Korea. Blumberg had asked Prince for his opinion on the significance of the physical appearance of the Australia antigen. The researchers had found they could concentrate this material by forcing it to the bottom of a tube in a rapidly spinning centrifuge. Because the antigen appeared as discrete particles, they examined its size and shape in an electron microscope. Prince thought it was a virus; Blumberg thought it was a large protein. Prince was sufficiently confident about the particles that he prepared a collaborative paper describing it as a new virus in human blood. Blumberg remained staunchly convinced of his own interpretation and declined to be a co-author. From then on Prince trod a separate path to knowledge of the virus, and ultimately to a separate style of vaccination against hepatitis B.

The mystery of Australia antigen emerged into the glare of popular attention. The central question was: is this Australia antigen actually a virus? Blumberg was in a difficult position to answer – self confessed in his woeful ignorance about viruses. Notwithstanding this unpromising start, amongst the staff were Irving Millman, an immunologist specialising in infectious diseases; W. Thomas London, a physician; and Manfred Bayer, an electron microscopist. They set about intense investigations of this material they insisted on calling Australia antigen. The hunt was on for answers to questions that grew ever more urgent the closer they got. What were its physical characteristics, its infectiousness and its immune reactions? Millman had joined the ICR in 1967 for opportunities to concentrate on immunology. He described his first day at ICR when he observed a colleague, Barbara Werner, setting up the process for concentrating the antigen from blood. Within two months Werner had developed hepatitis B, which she diagnosed by detecting the antigen in her own blood.

Millman suspected he was about to be pulled back into the field of infectious diseases, despite his reluctance to accept a viral nature of the material they were dealing with. They found these strange particles in astronomical numbers in blood. Millman could not imagine how a person could bear such load of a virus without dying. Viruses are so strange that many who work on them do not consider them truly alive. They are utterly dependent on the living cells which are the basic units of construction of the organisms they infect. Moreover, they manipulate the cells they infect into making many copies of the same virus, thus multiplying into a potentially catastrophic infection of the host animal or plant. All a virus needs to carry out this very special trick of parasitism is a core of a nucleic acid such as DNA, and a coat of several types of protein. Millman could find no nucleic acid in the Australia antigen; it seemed to be without the genetic means to replicate. These were still early days in the science of virology, of these infectious agents too small to see with an ordinary light microscope. Nevertheless, Millman remained confident enough to conclude the team was dealing with only part of a virus.

Thomas London had already published a paper on the problem of hepatitis occurring in people with kidney failure who attended a haemodialysis unit for cleansing their blood. He found the hepatitis was associated with Australia antigen and the study provided more epidemiological evidence that the antigen was associated with infection through contaminative routes. At last, the team had an explanatory framework upon which to pin a selection of their best information. What pattern would emerge? The appearance of the antigen particles was intriguing. Staining characteristics were as expected for a virus when prepared for observation in an electron microscope, but what the researchers saw were an oddly varied mix of spherical and tubular structures. Mostly on the small side for a virus, the spherical forms

gave the impression of hollowness in the grainy, monochrome, two dimensional images from the microscope. Hollow – how could that be? If this material consisted of one specific virus, why did it appear so varied and of such unlikely shapes?

The hunt for the virus of hepatitis B became a hot topic. Hints of evidence and inchoate ideas about such a virus had been developing for several decades and then, about 1970, common knowledge began to emerge from the collective intelligence. It was in Britain that the association of the antigen particles and a virus was first fully demonstrated, by D.S. Dane. Briefly he enjoyed the dubious honour of having the combined Australia antigen material and core of the hepatitis B virus named after him. He described the *Dane particle* in the serum of a patient with hepatitis who tested positive for Australia antigen with a new diagnostic method developed by Alfred Prince. Suddenly they all realized that the complete hepatitis B virus consists of a core containing the nucleic acid DNA. Around that is a layer of protein now known as hepatitis B core antigen, together with an outer layer known as hepatitis B surface antigen. This latter HBsAg is what was they still called Australia antigen, the hollow shells seen under the electron microscope. (Some virologists explain the prodigious amount of HBsAg produced by the virus as a special mechanism to evade the immunity of the infected person by swamping out the potential effect of protective antibody.)

Microbe hunting has a long and honourable history. Researchers such as Louis Pasteur in France and Robert Koch in Germany during the late 1800s devised basic procedures. Koch's set of postulates for proving that a particular disease is caused by a particular microbe was robust enough to still be taught to students of human and veterinary medicine. The team at the Institute for Cancer Research knew they had to satisfy these criteria. Could they find their possible virus in patients with the disease? Could they isolate it in laboratory culture? Could they transmit it back to animals and thus produce the same disease again? Their first difficulty was to culture the virus in some sort of bench-top system, such as broths containing cells of animals, or in lab animals such as mice. All their attempts failed. However, the Institute for Cancer Research had monkeys available for other work on possible viral causes of cancer. London and Millman used small groups of them to demonstrate that purified preparations of HBsAg caused signs of infection to develop.

Evidence for a viral cause of hepatitis B accumulated. If the preparations of Australia antigen were purified as much as possible, they were not infective, but they did induce an immune response in the inoculated animal. That is, the animal produced antibodies to the antigen. In contrast, less pure preparations were infective, producing signs of disease. It seemed that the antigen was associated with infection with the full virus that caused hepatitis. In contrast, the pure antigen was both non-infective and highly antigenic, that is it stimulated production of plentiful antibodies.

Small flashes began to pierce through the fog of investigation as germs of invention. Can we possibly use this plentiful antigen as a means of producing antibodies to protect people against infection? Can we convert this antigen from the blood of infected people into a vaccine to protect uninfected people? Crazy maybe, but the promise of a vaccine spurred them to intense effort. Vaccination is the holy grail of many workers on infectious diseases. Doctors have eradicated the dreaded smallpox virus from the world by vaccination and polio will soon be gone from us by the same means. A world without these fearful infections: what a goal! These microbes are fascinating once their agents are under a microscope but the

diseases are horrible to witness. Morbid fascination and the moral imperative to find some cure go hand in hand. Some researchers will retain faith in the robust public health approach, those with a gift for chemistry will tend toward the search for new drugs, but much of the glamour and money goes toward vaccines because of their history of success.

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Alfred Prince prepared a patent application for a vaccine, then withdrew because it was paired with a diagnostic test for hepatitis B and this test had rapidly been superseded. Saul Krugman in 1970 invented a vaccine against hepatitis B, consisting of serum from patients infected with hepatitis B, that was heat treated to kill the virus it contained. Researchers heard little more of these types of hepatitis vaccine, but the pressure to control the disease was now intensifying as better means were in sight.

Blumberg and Millman filed a patent for a vaccine in October 1969 and the US Patent Office granted it by January 1972. Included were images of gel plates that have an immediacy of the rush to get the discovery from the laboratory to the office. They were blotchy and murky, but sufficed for the application. The electron microscope images show the circular shape of the particles of antigen. The inventors went on to describe a process sufficient to produce a batch of vaccine that they could use experimentally, in the laboratory. Included were: prepared blood of people carrying hepatitis B virus; centrifugation at up to 370,000 times the force of gravity to separate the antigen particles; digestion of unwanted blood proteins and possible infectious agents with enzymes; a molecular sieving process; more centrifugation followed by a physical process to remove the solutes used during centrifugation; various options for further inactivation such as treatment with formalin preservative and pasteurisation; finally formulation into a material effective for injection as a medical vaccine. Sufficiently detailed it might have been for laboratory use, yet it was in no way a working protocol for the commercial manufacture of a vaccine that could be tested, legally registered, then sold for use in humans. The principle was established however: Blumberg and Millman had discovered a source of material and invented a method that was soon adapted to make a commercial hepatitis B vaccine.

The practical concern with hepatitis B, at that time and in countries such as America, was to prevent people contracting hepatitis B through blood transfusion. Screening blood supplies to reject those positive for HBsAg was now feasible with increasingly sensitive tests, for example the one developed by Prince. The increasing involvement of the team at the Institute for Cancer Research with this immediately practical work was in response to a change in the economic culture in America during the late 1960s. The halcyon days of ample staff and funds for free ranging science research during the post-war years were being ousted by competition with resurgent economies in Europe and Asia. Those scientists in the public sector sensitive to the winds of change soon recognized their need to operate like their peers in private companies: to apply and patent their research if possible.

Blumberg and his team knew their vaccine material could stimulate a strong immune response, but could it protect people against hepatitis B under ordinary living conditions? They knew insufficient about this material, or this disease; published research was very recent and much of it insufficiently corroborated. None of that was an impediment to their enthusiasm for this vaccine. Pragmatic opportunism had been the rule in early research on vaccination. Long before the causative organism of some diseases was known,

researchers were able to vaccinate against them successfully. The basic method of vaccination did not derive from an adequate understanding of the causation of the disease and of human immunology. Rather it derived from a style of research that owed more to the fact finding of natural history and the trial and error approach of early engineers rather than to what happens in a modern laboratory. Some of that gung-ho attitude still continued into the 1970s.

Merck and Company were in the business of inventing and manufacturing drugs and vaccines. Blumberg and team at the Institute for Cancer Research admitted they needed such a commercial partner. Maurice R. Hilleman worked for Merck developing, patenting and producing for sale vaccines against polio, influenza, measles, mumps and rubella. Hilleman had become a professional vaccinologist from an unpromising start. Born the youngest of eight children on small farm in Montana, his mother died days after the birth and his uncle reared him. He had a precocious interest in the books of Charles Darwin; he read them instead of listening to church sermons. These inspired him to a scholarship to study microbiology and chemistry at Montana State University, followed by doctorate studies on the question of whether *Clamydia* organisms were viruses or bacteria. He horrified his academic supervisors by insisting on directly entering industrial work for E.R. Squibb & Sons, where his first job was in development of vaccines against viruses threatening US troops in the Pacific area. His vaccine development continued at what is now known as the Walter Reed Army Institute of Research, thence on to Merck. On the farm he learnt to be bluntly pragmatic, at the Army Institute he learnt how to be effective at the top of a disciplined hierarchy, and from somewhere he acquired a rich vocabulary of profanities and obscenities. All of use when it came to hepatitis B vaccine.

Hilleman realized relations with the Institute for Cancer Research would be tricky to manage. Although Blumberg and Millman held the patent, it was assigned to the Institute as their employer. As a publicly funded institution, the ICR was constrained by federal rules on who could benefit financially from the manufacture and sale of a tangible product. (This was before the days of the Bayh-Dole Act that ameliorated these rules in favour of state funded institutions and universities.)

Furthermore, the nature of the vaccine was not only unique but had a deeply troublesome flaw. Since the source for this new vaccine could only be blood from people chronically infected with hepatitis B, Hilleman needed answers to some difficult questions. Who and where were these infected people? Could sufficient batches of blood be screened out of the supply for transfusions because they reacted positively in the new tests for hepatitis B? By then hepatitis B was becoming known as one of those contagious diseases with many routes of infection: blood transfusions; mother to unborn baby; blood-to-blood contact in scratches and cuts; sexual intercourse, specially between men; shared hypodermic needles of drug addicts. The disease was increasingly known as nasty and insalubrious. Could a purification process guarantee that all the virus of hepatitis B had been removed? And what about other agents of infection, even possible infections nobody knew about?

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In 1971 Merck offered the exploratory team from ICR a ready commitment for up to $5 million expenditure with a team of up to 10 people over the next five years to develop the patent toward production. An enticing offer, but obviously Merck needed to recover these costs then start to make a

profit, and for this they needed exclusive rights to the patent. These were early days for publicly funded institutions to be taking out patents and collaborating commercially. The ICR failed to apply for patent protection, therefore the intellectual rights became property of National Institutes of Health by default. The NIH proposed to license any approved companies who would pay a licensing fee to manufacture the vaccine. Merck and Co. were not interested in such an unfavourable arrangement and collaboration between ICR and Merck lapsed for five years.

In 1974 the Institute for Cancer Research combined with the American Oncologic Hospital to form the Fox Chase Cancer Center with a continuing remit for science research, but none for manufacture of vaccines. So a year later Blumberg and Millman went to Britain and France to talk business with potential manufacturers. Only Merieux expressed sustained interest and this company, in collaboration with the Institut Pasteur in Paris, actually became the first to market a commercial hepatitis B vaccine, under the name Hevac B Pasteur, just a few months before the Merck vaccine was released in 1982. By this time, the ICR had obtained several foreign patents to the vaccine, and the US government proposed that ICR could act as an agent to license the patent in such a way that they had control over both domestic and foreign rights. Thus the US government would remain the owner of the original patent of Blumberg and Millman, while all foreign patents would remain the property of ICR. In August 1975 the parties reached an agreement: Merck would obtain all foreign patent rights from ICR and they would be able to obtain the licence from the US government for the domestic rights on a non-exclusive basis. A dismal bureaucratic delay, as many people in the public sector struggled to adapt to rapidly changing economic forces.

Maurice Hilleman, pragmatist and committee of one, directed his team to develop techniques for a concentration and purification process that would solve the problems of limited source of supply direct from human blood and of potential contamination. Blumberg's patent pointed in the right direction but Hilleman was unimpressed with the technical competence of that patent. As he later exclaimed to his biographer: 'Somebody had actually issued a patent for that crap.' What most worried Hilleman was how to destroy any of the uniquely tough agent of the incurable and fatal Creutzfeld-Jakob disease that might contaminate the source blood. This was in the days before the existence of the human immunodeficiency virus in America was known. Autocratic as Hilleman was he still needed to prove to his managers at Merck that this vaccine was feasible and profitable on an industrial scale, and he needed to prove to government agencies that the vaccine would be safe for use.

He was determined on a belt-and-braces approach. From blood the vaccinologists extracted plasma, which is equivalent to serum. Then they treated the plasma to precipitate out the particles of HBsAg before partially purifying the precipitate in ultracentrifuges. These are scary machines built to operate at more than half a million times the normal force of gravity, at which state the phrase 'high-tech hum' is sensed to mean not just a sound but another world of unimaginable forces. Following this bulk separation of the particles they treated them with both an enzyme and urea to kill all microbes known at that time. Then they filtered the material through a chemical gel acting as a molecular sieve. As if that was not cautious enough, they added formalin to kill any microbes that might still have escaped all these assaults. Hilleman calculated the probability of any microbe escaping through each step to be one in one hundred thousand. Multiplying these minute fractions together lowers the risk toward infinitely small. But still it was a nerve wracking business even for a trojan like Hilleman, who inevitably had to hand over his

laboratory process to the vaccine manufacture division of Merck. They tried to economise on his perfectionist protocol. ‘Goddamn meatheads are everywhere’ he cursed.

Hilleman tested preliminary laboratory batches of the vaccine on middle managers at Merck. They were somewhat reluctant but Hilleman was not a man to say ‘No’ to and the consent forms he issued to them had only one option: agreement. Of course, he tested it on himself, being a proper microbiologist. Soon he needed someone capable of organising a full scale clinical trial to demonstrate sufficient protection of people at high risk. Hilleman was lucky to have a kindred spirit readily available. Wolf Szmuness worked on infectious diseases at the New York Blood Center. As a young doctor he learnt about public health in a labour camp in Siberia. He had found himself imprisoned there after fleeing eastwards from western invaders advancing on Warsaw, but was later able to establish himself as an epidemiologist with a degree from the University of Kharkiv, in Ukraine. In the late 1960s he defected from communist dominated Poland to America, and, starting as a laboratory technician in the Blood Center, soon worked his way to a full position with Columbia University. Moreover, Szmuness had a score to settle with hepatitis B: his wife had nearly died of liver disease caused by the virus.

His test of the vaccine involved 1083 people over two years. Placebo-controlled, randomised, double-blind is the jargon for the standard trial he used. All the volunteers were randomly divided into two equal groups; those in one group received the full vaccine; those in the other received a mimic of the vaccine as a placebo with no active ingredient. The volunteers were not told if the inoculation was vaccine or placebo. The results of the vaccination were assessed by researchers different from those who had inoculated the vaccine and placebo and these researchers did not know the group of any individual person they were assessing. Louis Pasteur and Robert Koch would have approved.

Szumness the epidemiologist knew that in America, the percentage of people infected with hepatitis B at a specific date was low, it had a low prevalence. To gather easily a sufficient sample he had to seek people with unusually high prevalence. Szumness the pragmatist asked the help of gays in New York – genuine volunteers. The researchers were delighted when they opened the results and matched them to reveal that within two months of the start of the trial, 77 percent of the men vaccinated had developed high levels of antibody against HBsAg. After the booster dose, this rose to 96 percent and remained high for the rest of the trial. Of those men in the trial who received the vaccine only 1.4 to 3.4 percent developed symptoms of hepatitis B infection compared to 18 to 27 percent in those who received the placebo. By 1983, Hilleman could report that over 20,000 people had received the vaccine in various clinical trials without untoward effects, whilst by 1982 the Federal Drugs Administration had already approved the vaccine for commercial sale.

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Another implication surrounding the role of serendipity in research and invention is the eureka moment, the unique breakthrough leading promptly to the wondrous invention, as the process is so often portrayed. What if Baruch Blumberg had not gone to Oxford, met Tony Allison and been persuaded to change from research on arthritis to polymorphisms in blood proteins? Would there now be a vaccine against hepatitis B? Yes, because Saul Krugman had already devoted so much of his working life to controlling hepatitis that he went as far as an experimental vaccine by 1970, even though he knew nothing of the Australia

antigen. If he had been working in less controversial circumstances it would not have taken Krugman or fellow workers long to find the HBsAg in blood plasma. Similarly, if both Blumberg and Krugman had worked in different areas then virologist Prince and his colleagues working in a blood transfusion clinic would soon have been the first to be credited with the discovery of the nature of hepatitis B and the invention of the vaccine.

Yes, there would be a vaccine because the deeper probings of more technically versatile research, particularly in those countries most severely affected by hepatitis B, would inevitably have revealed HBsAg. Unknown and unexpected, nevertheless this material existed in massive amounts in the blood of chronic sufferers. So its antigenic nature would soon have been discovered because so much medical research uses reactions between unknown antigen and test antibody.

Once researchers knew the source and characteristics of the antigen they could confidently predict manufacture of a vaccine following well established generic procedures. They achieved all of this only a few years after the disease was finally proved to be caused by a virus and whilst scarcely anything was known about the molecular biology or immune reactions provoked by this virus. Again, Blumberg's typical frankness tells the inside story: 'Oddly, most of the important applications of the research on HBV were realized before there was a significant understanding of the virus's molecular details.' Others had been equally frank long before. The Roman medical writer, Aulus Celsus, from the first century AD: 'That medicines and cures were first found out, and then after the reasons and causes were discoursed; and not the causes first found out, and by light of them the medicines and cures discovered.' Science researchers in the medical business make jokes amongst themselves, from their ivory towers, about the concept of evidence based medicine that is now promoted by their clinician colleagues. The clinicians meanwhile have patients in need of help.

The original plasma vaccine against hepatitis B was invented by combining a discovery with 'Prior Art', as the phrase used in patent applications goes. The methods in the original patent of 1972 describe briefly a series of adaptations to existing methods. Many years of hard toil were spent adapting these methods to a vaccine fit for purpose, but most of that involved systematic trials of varying conditions above and below starting conditions that would have derived more from personal experience and knowledge of the frontier literature than calculations based on laws of nature. This was innovation from a base of pre-existing technology for which organisational skills were as important as a talent for discovery. What made this improvement unique and patentable was discovery of the essential antigen, followed by the inspired detective work that crucially led to understanding the antigen as a part of the hepatitis virus which could be made into a vaccine.

Whilst the plasma vaccine was being developed and tested there appeared in a 1973 issue of the journal *Nature* an editorial with the title 'Synthetic hepatitis B vaccine?' After closer reading an intrigued commercial vaccine researcher would have tracked down the original authors who reported preliminary studies on the possibility of stringing together the amino acid molecules that constitute proteins in order to mimic HBsAg sufficiently well for the synthetic protein to be used in a vaccine. Such a vaccine would be susceptible to the economies of scale, use relatively cheap raw materials, and be free of biological contamination that was such a hindrance to human plasma as a source of vaccine. A vaccine with those characteristics was soon invented, but through a completely separate technical route. That development is

parallel to the story of synthetic insulin and was made possible because the original plasma vaccine was an obvious early subject for the new technology. Thus all the more, the new synthetic vaccine enhances the extraordinary achievement of the developers of the original vaccine who, during their open minded enquiries into the natural world, found something totally unexpected and converted it into a vaccine that protected people against both an insidious virus and a cancer.

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