Debilitating and deadly, hepatitis has plagued humankind since the beginning of recorded history. But the course of this disease was irrevocably changed with the accidental convergence of a medical researcher curious about why some people are especially prone to various ailments, another medical researcher wondering why people often become sick after receiving blood transfusions, and the blood of an Australian aborigine. That convergence led to a discovery that in less than a decade spurred a blood-screening campaign that dramatically reduced the incidence of hepatitis spread by blood transfusions—hapatitis B. The discovery also led to a highly effective hepatitis vaccine that not only introduced a novel way of protecting people from infectious diseases but also is the first effective vaccine against liver cancer. Yet the scientists whose work revolutionized the study of hepatitis did not even have the disease in mind when they embarked on their investigations. As often happens in science and medicine, the landmark discovery grew not out of “targeted research” but from studies aimed at answering more fundamental questions about nature. The following article, adapted in part from an account by researcher Baruch Blumberg, who shared the 1976 Nobel Prize for physiology or medicine, explores the trail of research that led to the discovery of many of the viruses that cause hepatitis and to blood screening for and revolutionary vaccines against some of them. It provides a dramatic example of how science works and how basic research can lead to practical results that were virtually unimaginable when the research was done.

Hepatitis B: A Debilitating Disease

Viral hepatitis is one of the most common infectious diseases, causing an estimated 1.5 million deaths worldwide each year. The distinctive yellow jaundice that hepatitis B usually imparts to its victims’ skin has made it an easily detectable disease throughout recorded history. Other telltale signs of the acute disease are fever, chills, fatigue, nausea, loss of appetite, and abdominal pain. Symptoms usually subside after several weeks, although some people experience a severe form of hepatitis B that is rapidly fatal.

The acute disease is not the only way that hepatitis B afflicts humans, however. Some people with chronic hepatitis do not experience acute symptoms but may lose weight, feel tired, have abdominal pain and jaundice, and experience liver damage. In these cases the disease continues to damage more of the liver over a period of 15 years or more, until death ensues prematurely from liver failure or liver cancer. Also, a large number of people worldwide are “carriers,” meaning their immune systems

A 1988 poster issued by the Guam Dept. of Public Health and Social Services urges mothers to get their children immunized against hepatitis B. More than 85 countries, including the United States, have adopted universal childhood vaccination for the disease. (Visual Image Presentations)
tolerate the virus and do not see it as foreign. Carriers thus do not have any symptoms for many decades but can unwittingly infect other people. Carrier mothers very often transmit the virus to their newborn children, who themselves will become carriers because the virus is treated as a natural part of their bodies.

Although hepatitis has been known for centuries, before World War II doctors did not know that it was caused by a virus. It was assumed to be contagious because epidemics of hepatitis often occurred in crowded, unsanitary conditions, but how it was passed from person to person was a mystery.

Headway into solving the mystery was made in the 1940s by a British doctor, F. O. MacCallum, who specialized in liver disease. He was concerned not so much with hepatitis as with the extremely deadly yellow fever transmitted by mosquitoes, which was killing soldiers in Africa and South America. Charged with the production of a yellow fever vaccine, MacCallum was perplexed as to why a sizable proportion of soldiers who received the yellow fever vaccine developed hepatitis a few months later. The yellow fever vaccine contained human serum, and MacCallum was aware of other hepatitis cases reported in the medical literature that followed inoculation with vaccines containing human serum. He also knew of cases that followed the reuse of unsterilized syringes and needles in the treatment of diabetes or venereal disease, instruments that could contain particles of blood. MacCallum came to suspect that a virus carried in human blood could cause hepatitis.

A series of observations of volunteers by MacCallum and others during and shortly after the war strengthened that hypothesis and made it clear that hepatitis can also be spread by other means than through blood. MacCallum coined the terms hepatitis A for the form of the disease that is spread primarily through food and water contaminated with minute quantities of fecal material and hepatitis B for the form that is transmitted mainly by exposure to contaminated blood.

### Searching the Blood for Clues

During the next decade and a half, researchers at many laboratories tried in vain to isolate the infectious agents that cause the two types of hepatitis. Scientists suspected that the culprit organisms were viruses because they were small enough to pass through some of the smallest-pore filters used in experiments, but the scientists were unable to grow them in order to identify and study them. By the mid-1960s, hepatitis research had reached a discouraging deadlock. Then a remarkable advance in knowledge of the causes of hepatitis was made by someone who was not working on the disease at the time. Baruch Blumberg, a medical researcher specializing in internal medicine and biochemistry, was interested in a more basic question—why were some people prone to particular diseases?

As a medical student in the early 1950s, Blumberg had conducted research in Surinam on elephantiasis, a parasitic disease common in the tropics. His investigations showed that some of the ethnic populations in the town in which he worked were more susceptible to elephantiasis than others, even though everyone was apparently exposed to the same conditions. A few years later he began to suspect that differences in susceptibility stemmed from variations in the genetic makeup of different ethnic populations, but the tools of modern molecular biology that now allow scientists to link disease susceptibility to variations in genes had not yet been invented. At the time, researchers trying to detect genetic differences that might be tied to disease susceptibility looked for inherited differences in specific blood proteins. These differences, called polymorphisms, were in some cases assumed to be maintained over generations because they gave those who carried them a survival advantage, such as resistance to a disease.

Researchers had already discovered a number of polymorphisms in blood proteins—for example, the different blood proteins that determine type A, O, or B blood—but this field was a vast and relatively unexplored terrain that promised to unlock the secrets of disease susceptibility. In the late 1950s, Blumberg embarked on research aimed at finding new polymorphisms in blood proteins. To that end he began collecting blood samples from populations all over the world.

In the early 1960s, Blumberg was at the National Institutes of Health (NIH), where he collaborated
with biochemist Anthony Allison on a way to detect novel blood proteins quickly and easily. The scientists reasoned that patients who received multiple blood transfusions had probably encountered blood proteins sufficiently different from their own to prompt their bodies to generate an immune reaction, or antibodies, against the foreign proteins, or antigens. They used a technique known as agar gel diffusion, which relies on the immune system’s ability to spot minor differences in proteins and to produce an antigen-antibody interaction in response to a novel blood protein.

Agar gel diffusion involves the migration of proteins and antigen-antibody complexes through gels. This technique detects the immune system’s ability to spot minor differences in proteins and novel antigen-antibody interactions. First the researchers coated glass slides with a gel, in the center of which they placed some serum from a patient who had received many transfusions. That sample was then surrounded by gel containing sera from normal people who had not received transfusions. All the serum samples diffused slowly through the gel. If any components of the normal people’s sera reacted with antibodies from the patient’s blood sample, a telltale white line appeared, indicating the presence of a combination of antigen and antibody in a concentration large enough to be detected. This reaction had two possible implications: one, that the transfused patient’s blood contained antibodies that had been exposed before to antigens in the other people’s sera; and two, that material found in one person’s serum might be sufficiently foreign to be an antigen to another person.

**Breakthrough Blood Sample**

Meanwhile, reactions to someone else’s blood were also of interest to blood specialist Harvey Alter at the NIH Blood Bank. Alter wanted to find out why some patients developed fever, chills, or rashes after blood transfusions. He thought they might be suffering from immune reactions to foreign proteins (antigens) in donated blood. When Alter learned that Blumberg was looking for immune reactions in the blood of patients who had received many transfusions, he went to see him, and they decided to collaborate. Blumberg and Alter used agar gel diffusion to test sera from patients who had received multiple transfusions (for example, hemophilia and leukemia patients) against panels of serum in Blumberg’s international collection from people of widely varied origins. In 1963, after months of experiments, the researchers discovered that serum from a New York hemophilia patient reacted with serum from a person residing in the opposite corner of the world—an Australian aborigine. This finding was not unusual in itself; up to that point, the transfused patients’ blood in these experiments had reacted with high frequency to other sera, indicating that the patients had been exposed to many common antigens through transfusions. As a result, though, it had not been possible to draw any definitive conclusions as to which antigen or antigens were causing the reaction—until now. It turned out that in the particular experiment with the Australian aborigine’s serum, only one of 24 hemophilia patients’ sera reacted with it. The significance of this was exciting, for it implied that a single and rare antigen was causing the reaction—until now. It turned out that in the particular experiment with the Australian aborigine’s serum, only one of 24 hemophilia patients’ sera reacted with it. The significance of this was exciting, for it implied that a single and rare antigen was causing the reaction. So what was the antigen? Since it occurred only rarely, it was unlikely to be an antigen caused by genetic variation in human blood. Instead, it was more likely to be from an infectious source.

Intrigued by this question, although still not working on hepatitis B directly, Blumberg and Alter tested the serum of the hemophiliac in question against...
thousands of serum samples. They found that samples from only one in 1,000 healthy nonhemophiliac American blood donors reacted with the hemophiliac’s serum, whereas samples from one in 10 of the leukemia patients reacted. Whatever antigen in the Australian aborigine’s blood had caused the reaction in Blumberg and Alter’s tests was also found often in the blood of leukemia patients. Moreover, the antigen was rarely found in normal patients’ blood but frequently in hemophiliacs and leukemia patients. The researchers labeled the mysterious protein Australian antigen (Aa) in reference to the homeland of the aborigine whose blood led to its discovery. They hypothesized that an unknown antigen in the Australian aborigine’s blood was reacting with antibodies in the blood of certain hemophilia and leukemia patients.

**Surprising Finding**

Blumberg thought he might have detected an inherited blood-protein polymorphism that affected people's susceptibility to leukemia, but he knew that other possibilities (including an infectious agent like a virus) might explain the link between Aa and leukemia. To clarify that link, he began searching for Aa in the blood of children with Down syndrome, who run a particularly high risk of developing leukemia. Almost one-third of these children had Aa. Blumberg then tested Down patients of various ages who were housed in various settings. Newborn patients tested negative for Aa, but the bigger the institution in which the patient resided, the more likely that he or she tested positive. This suggested that Aa might be linked to an infection of some sort.

Usually, the children who tested negative for Aa remained negative when retested and those who tested positive remained positive, as expected for a blood-protein polymorphism. But in 1966, Blumberg, W. Thomas London, and Alton Sutnick discovered that a 12-year-old boy with Down syndrome who had no trace of Aa in his serum when he was first tested showed presence of the antigen in his blood a few months later. Significantly, this boy not only displayed Aa by the agar gel diffusion test but he also had hepatitis. The coincidence suggested that, rather than being associated with an inherited blood-protein polymorphism, Aa was linked to hepatitis. Immediately researchers began exploring this hypothesis. In testing patients with and without hepatitis, they found that those with hepatitis tested positive for Aa more often than those without the disease. The hypothesis was dramatically bolstered when Blumberg’s laboratory technician began to feel ill. Aware of the link between Aa and hepatitis, she tested her own serum for the presence of Aa—and found it positive. She later developed hepatitis and became one of the first people

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**The Hepatitis B Story**

*This timeline shows the chain of basic research that led to development of the hepatitis B vaccine and subsequent tests for other hepatitis viruses.*

- **2000 B.C.**
  First recorded references to hepatitis epidemics.

- **1963**
  Baruch Blumberg and Harvey Alter discover Aa, the Australian antigen (later called HBsAg).

- **1947**
  F. O. MacCallum, using human volunteers, differentiates hepatitis A, which is spread by contaminated food and water, from hepatitis B, which is spread by blood.

- **1967-1968**
  Blumberg, Kazuo Okochi, Alfred Prince, Alberto Vierrucci, and colleagues report that Aa is involved in the development of hepatitis B.

- **1969**
  Irving Millman and Blumberg devise a concept and through the Fox Chase Cancer Center receive a patent for using Aa to prepare a hepatitis B vaccine.

- **1970**
  D. S. Dane discovers whole hepatitis B virus particles in blood samples examined with an electron microscope.

- **1972**
  Laws are passed in the United States requiring testing of donor blood for HBsAg antigen.

- **1973-1974**
  Stephen Feinstone and colleagues and Maurice Hilleman and colleagues discover and describe hepatitis A virus.

This article was published in 2000 and has not been updated or revised.
whose viral hepatitis was diagnosed with the Aa test. Hearing of Blumberg’s findings, virologist Alfred Prince of the New York Blood Center started an experiment in the mid-1960s that would eventually confirm the link between Aa and hepatitis. Knowing that at least one in 10 patients who received multiple blood transfusions would come down with hepatitis, Prince wanted to determine whether Aa appeared in the blood during the incubation period of the disease, before any symptoms of illness, as would occur if Aa were part of the virus that caused the hepatitis. Prince began taking blood samples from certain patients at the New York Blood Center at regular intervals and storing them in a freezer. Finally, in 1968, he heard that a patient whose blood he had collected had developed clear symptoms of hepatitis. When he tested the man’s blood samples, he found no evidence of Aa in the early batches but clear evidence of it in blood taken a few weeks before onset of the illness. Such seemingly direct evidence strongly suggested that Aa was indeed involved in the development of hepatitis B.

At about the same time, University of Tokyo’s Kazuo Okochi showed that blood that tested positive for Aa was much more likely to transmit hepatitis to transfused patients than blood that tested negative. Alberto Vierrucci, of the University of Siena, Italy, independently confirmed Prince’s and Okochi’s reports in the same year, 1968. Further strengthening the link between Aa and hepatitis were discoveries made with an electron microscope in 1970 by D. S. Dane and colleagues at Middlesex Hospital in London and K. E. Anderson and colleagues in New York of what looked like virus particles in the sera of people who tested positive for Aa. They also found particles in the liver cells of patients with hepatitis.

By the end of 1970, mounting evidence led nearly everyone in the field to the same conclusion: Aa was part of the virus that causes hepatitis B. (At this point nomenclature for Aa was changed to HAA, or hepatitis-associated antigen; it is now officially called HBsAg, for hepatitis B surface antigen.) The leukemia and hemophilia patients whose blood showed a high incidence of HBsAg all had needed frequent transfusions and therefore were more likely to have received blood contaminated with hepatitis B virus.

The HBsAg-hepatitis B discovery had stunning clinical implications. In the United States in the 1960s, a large percentage of donated blood was obtained from paid donors, who were more likely than the general population to have hepatitis B. As a consequence, the incidence of posttransfusion hepatitis was high; in some studies the disease developed in half the patients who received large numbers of transfusions for extensive...
surgical treatments. The medical community recognized that it could dramatically reduce posttransfusion hepatitis if it could screen HBsAg-contaminated blood by an appropriate test.

But the gel diffusion technique that Blumberg and Alter used to detect HBsAg in blood was not sufficiently sensitive for accurate blood screening. Fortunately, the curiosity of two researchers at the Bronx Veterans Administration Medical Center as to what happens to insulin in the blood of diabetics had led in the early 1950s to a revolutionary technique for detecting and measuring tiny amounts of serum proteins and antibodies. Rosalyn Yalow and Solomon Berson had been perplexed as to how it was that diabetics produce insulin, a hormone produced by the pancreas, even though diabetes is characterized by symptoms that indicate a lack of insulin. To determine what happens to insulin in diabetics once it enters the bloodstream, they prepared a radioactive form of the hormone that could be easily detected. However, while studying the blood of diabetics who had received injections of radioactive insulin, the researchers discovered that the insulin was binding to antibodies generated by the patients’ immune systems. That discovery led Yalow and Berson to devise a technique called radioimmunoassay, which can trace minute quantities of a substance as it binds to an antibody or other protein. Not only was it simpler than gel diffusion techniques, the radioimmunoassay was also a thousand times more sensitive. For her development of the radioimmunoassay, Yalow shared the 1977 Nobel Prize for physiology or medicine.

Several commercial companies and academic researchers adapted the radioimmunoassay to produce kits for the accurate detection of HBsAg in blood. In the United States, laws were passed in 1972 requiring that donated blood be tested for hepatitis B virus (HBV). As a result, all blood banks tested every sample of blood, and posttransfusion hepatitis due to hepatitis B became a rarity. Screening of donated blood for HBV has produced an estimated savings in medical treatments of some half-billion dollars a year in the United States alone.

What About Those Particles?

The benefits of the HBsAg/hepatitis B discovery soon extended beyond protecting people who received blood transfusions from hepatitis B to the broader arena of protecting all people from the disease. In the late 1960s, Blumberg, working at the Fox Chase Cancer Center (FCCC) with immunologist and virologist Barbara Werner, electron microscopist Manfred Bayer, and molecular biologist Lawrence Loeb, described further the small particles isolated from HBsAg-positive blood and visualized with the electron microscope. Some particles were whole viruses; others were shown to contain no nucleic acid—the gene or genes responsible for causing infection and disease.

Several experiments showed that the particles could induce protective immunity. In 1971, infectious disease expert Saul Krugman, of New York University, published a paper on the accidental discovery that injections of hepatitis B-contaminated blood that had been heated to kill viruses gave some protection against hepatitis B. Although the nucleic-acid-free particles Blumberg isolated could not cause disease, several findings suggested they could be used to stimulate immunity against the infectious virus. Okochi and colleagues found that patients who had received transfusions and whose blood contained antibodies to HBsAg were less likely to develop posttransfusion hepatitis than were patients without the antibody.

Intrigued by the notion that HBsAg provokes an immune response that protects people from hepatitis B, Blumberg and Irving Millman, working at FCCC, proposed that a vaccine could be made from HBsAg particles obtained from the blood of hepatitis B carriers. This was an unusual approach to developing a vaccine. Before 1969 all vaccines were made in one of three ways. In one method they were prepared from whole viruses or bacteria that had been killed to prevent infection. In another they were made from weakened strains of pathogenic organisms that caused...
mild or no symptoms when injected as a vaccine yet protected recipients from more severe wild strains. Vaccines had also been made from whole viruses that, while not causing disease themselves, were closely related to viruses that did. But no vaccines had been made from human blood using only parts, or “subunits,” of human virus. FCCC filed a patent for a method involving this concept in 1969.

Maurice Hilleman and colleagues at the Merck Institute for Therapeutic Research recognized the importance of the possibility of developing a vaccine from particles, or subunits, of the virus. In 1971, Merck, where scientists were independently working along related lines, took a license from FCCC and, after many years of extensive research and testing, developed a subunit hepatitis B vaccine made from HBsAg purified from blood. In 1980, Wolf Szmuness, of the New York Blood Center, and colleagues at Merck showed that the vaccine provided more than 90 percent protection against hepatitis B and had no adverse side effects. In 1981, the serum-derived subunit vaccine was made available for general use.

In an independent line of basic research in animals, a group of scientists led by Howard Bachrach at the U.S. Department of Agriculture reported in 1981 the first effective protein vaccine for use in animals or humans. His work resulted in the first viral protein vaccine, against foot-and-mouth disease.

Production of the hepatitis B subunit vaccine in large quantities was hampered by the need for the blood of hepatitis B carriers and the realization that such blood could be contaminated with other viruses. Building on an interest in this problem, William Rutter and colleagues at the University of California-San Francisco in 1977 obtained material containing the virus from Merck. They proposed to develop a hepatitis B vaccine by preparing HBsAg particles using recombinant technology. This new process would both ensure no contamination from other sources and allow production of large quantities of the vaccine.

The concept of producing a vaccine in this way was totally new. After cloning the hepatitis B virus and obtaining the genetic sequence of HBsAg, Rutter and colleagues explored a variety of different biological systems in which to produce the particles using recombinant techniques. They were unsuccessful using bacteria. Then, in 1980 and 1981, Rutter collaborated with Benjamin Hall and colleagues, of the University of Washington, who had developed a model system using yeast cells. Rutter and Hall successfully produced pure HBsAg particles from genetically altered yeast cells. Rutter and colleagues then founded Chiron Corporation, in part to develop the HBsAg vaccine through a contractual relationship with Merck and also to develop other medical therapeutics using recombinant techniques. At Merck, Hilleman used the recombinant yeast-derived HBsAg, rather than blood plasma-derived antigen, to make an improved version of a hepatitis B vaccine. This recombinant vaccine was the first of its kind for use in humans and was licensed by the U.S. Food and Drug Administration for general use in 1986, after nine years of research.

Further studies have revealed that hepatitis B can be passed from person to person not only through blood but also through sexual contact or from a carrier mother to her newborn child. An important study in Taiwan by Palmer Beasley and colleagues in 1975 showed that nearly two-thirds of infants born to HBsAg-positive women became HBsAg carriers themselves. The hepatitis B vaccine protects people from all forms of transmission. Because infants or children infected with hepatitis B virus have an extremely high risk of becoming lifelong carriers of the disease, universal childhood vaccination for hepatitis B has now been adopted by more than 85 countries, including the United States.

**A Vaccine to Prevent Liver Cancer**

Liver cancer is one of the most prevalent cancers in the world and the most common cancer in some parts of Asia. Because it is typically not detected until the disease is in an advanced stage, liver cancer is usually fatal within a year of diagnosis. More than 60 percent of liver cancers worldwide have been linked to hepatitis B, and one study has shown that chronic carriers of the hepatitis virus are about 100 times more likely than noncarriers to die of liver cancer. The hepatitis B vaccine thus can not only prevent deaths from hepatitis B but also holds promise for substantially preventing deaths from liver cancer. Studies have shown that hepatitis B vaccination programs caused the number of hepatitis B carriers to decrease substantially in some communities. Although more long-term studies are needed, one 10-year study in Taiwan found that use of the hepatitis B vaccine reduced the HBsAg carrier rate in children from 10 percent to less than 1 percent. Researchers anticipate that this significant decrease will be linked to a lower incidence of liver cancer in children.
ABCs of Hepatitis Revealed

Encouraged by successful pinpointing of the hepatitis B virus, many researchers pursued research aimed at learning more about the hepatitis A virus as well as other suspected hepatitis viruses. In 1973, Stephen M. Feinstone and colleagues at NIH used an electron microscope to visualize viral particles in the stools of infected individuals. At about the same time, Hilleman and colleagues at Merck defined and characterized the human hepatitis A virus that Feinstone had purified from the infected livers of marmosets, a type of monkey. By 1996, Hilleman and his colleagues had made an attenuated hepatitis A vaccine (that is, a vaccine made from a virus that is modified in such a way that it cannot cause disease) that was licensed for general use. Another hepatitis A vaccine was developed by SmithKline Beecham Laboratories.

In 1978, Italian gastroenterologist Mario Rizzetto and molecular virologist John Gerin, of Georgetown University, discovered the delta, or hepatitis D, virus. This rare virus depends on hepatitis B to survive and in combination with hepatitis B causes a much more severe form of the disease. In 1983, Mikhail Balayan of the Institute of Poliomyelitis and Viral Encephalitides in Moscow discovered hepatitis E virus. Like hepatitis A, hepatitis E is spread by contaminated food and water and is usually found during localized epidemics.

Despite blood screening for hepatitis B, some patients still came down with posttransfusion hepatitis due to what was termed “non A-non B” hepatitis. Scientists suspected that yet another virus or viruses could be transmitted via blood and turned their attention to developing strategies first to isolate non A-non B hepatitis and then a test to identify it in blood.

After reaching those milestones, they hoped to someday work toward developing a recombinant vaccine. But the non A-non B hepatitis agent proved especially elusive. In 1983, Chiron Corporation began supporting a large research program to solve the puzzle, involving a collaboration between Daniel Bradley at the Centers for Disease Control and Prevention and Michael Houghton, George Kuo, and Que Lim Choo and colleagues at Chiron. Bradley, who had been studying chimpanzees infected with human serum containing non A-non B hepatitis agent or agents, provided contaminated chimpanzee sera to Chiron. In 1989, Michael Houghton and colleagues ushered in a new era for the discovery of infectious agents when they used molecular biological techniques to clone hepatitis C, the agent responsible for 80 to 90 percent of non A-non B hepatitis. This was a scientific tour de force because the unknown agent, unlike the other hepatitis viruses identified up to that point, had not been visualized, grown in culture, or immunologically defined. Following the introduction of sensitive and effective blood tests for the detection of hepatitis C in 1990, the risk of transfusion-related hepatitis is now in the range of one in 100,000 units transfused.

The discovery in the past 30 years of these hepatitis viruses and promising developments in blood screening and vaccines lead researchers to hope that viral hepatitis will soon be controlled and will no longer be the threat to human health it has been for thousands of years.

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