

**Unit 2: Lesson 4 – Vaccine History and Research****Vaccinated: One Man’s Quest to Defeat the World’s Deadliest Diseases**  
**Chapter 3: Eight Doors**

Paul A. Offit, M.D. © 2007, HarperCollins Publishers, Inc., pp. 31-43.

*“If I have seen farther than others, it was because I was standing on the shoulders of giants.”*

– Sir Isaac Newton

What possessed Maurice Hilleman to take his daughter’s mumps virus and inject it into hen’s eggs and minced chick embryos? Why did he cut off the chicks’ heads before using them? And most importantly, why in the 1960s did he resort to a process so seemingly crude, arcane, and convoluted? Eight critical experiments performed during the previous century determined Hilleman’s choices.

From Edward Jenner, Hilleman learned the power of vaccines. Jenner’s vaccine eradicated mankind’s deadliest infection, smallpox, from the face of the earth.

Easily spread by tiny droplets of saliva containing millions of virus particles, smallpox was a common, severe, debilitating infection. The virus caused high fever and a permanently disfiguring, pus-filled rash with a smell reminiscent of rotting flesh. Smallpox killed one of every three of its victims and blinded many survivors. In 1492, when Christopher Columbus crossed the Atlantic Ocean, 72 million Indians lived in North America; by 1800, only six hundred thousand remained. Smallpox—brought by European settlers—killed most of the rest. Indeed, smallpox has killed more people than all other infectious diseases combined.

In 1768, when Edward Jenner was thirteen years old and training as an apprentice apothecary in Chipping Sodbury, England, he approached a young milkmaid who appeared ill. “Are you coming down with the smallpox?” he asked. “I cannot take that disease,” she said, “for I have had the cowpox.” Cowpox was a disease that caused blisters on the udders of cows. Sometimes people who milked cows with cowpox would get these same blisters on their hands. Jenner was only a boy, so he didn’t give much thought to the milkmaid’s notion of what prevented diseases. But Edward Jenner remembered that conversation for the rest of his life.

Years later, while training in London, Jenner told the famous surgeon John Hunter about the milkmaid’s observation. Hunter encouraged Jenner to test the theory. “Don’t think, but try,” said Hunter. “Be patient, be accurate.” On May 14, 1796, several months before George Washington gave his farewell address, Edward Jenner got his chance. Sarah Nelmes, a milkmaid in Jenner’s employ, developed cowpox blisters on her hands and wrists. Jenner removed the pus from one of the blisters and injected it into the arm

of James Phipps, the eight-year-old son of a local laborer. Six weeks later, Jenner injected Phipps with pus taken from a case of smallpox “in order to ascertain whether the boy, after feeling so slight an affection of the system from the cowpox virus, was secure from contagion of smallpox.” Typically inoculation with smallpox caused high fever; chills; an ulcerating, painful rash; and occasionally death. But nothing happened to James Phipps. Later, Jenner injected Phipps twenty more times with pus from people with smallpox; each time Phipps survived without incident. Apparently, cowpox virus was similar enough to smallpox so that inoculation with one protected against disease caused by the other.

Two years later, Jenner published his observations under the lengthy title “An Inquiry into the Causes and Effects of Variolae Vaccinae, a Disease Discovered in Some of the Western Counties of England, Particularly Gloucestershire, and Known by the Name of Cow Pox.” Jenner used the term *variolae vaccinae*—literally “smallpox of the cow” and later the source of the word *vaccine*. Within one year of his publication, physicians had inoculated a thousand people with cowpox and translated Jenner’s observations into several languages. It took about two hundred years for Jenner’s vaccine to eradicate smallpox from the face of the earth. (Although the disease is gone, the virus isn’t. Fearing that smallpox virus would be used as a weapon of terror, the U. S. government supported a short-lived program to immunize hospital workers in October 2002, five months before the invasion of Iraq.)

Despite Jenner’s success, scientific advances often come with a price—the landscape of vaccines is littered with tragedy. Jenner lacked a reliable, consistent, and continual source of cowpox virus. So he inoculated cowpox under the skin of a volunteer, waited eight days until it caused a blister, removed the pus, and inoculated it into the arm of the next person. Many children were vaccinated using this arm-to-arm technique. For example, in St. Petersburg, Russia, in 1801 a recently vaccinated girl was sent to a local orphanage to serve as a source of cowpox virus for other children. The orphanage continued arm-to-arm inoculation for more than ninety years. But arm-to-arm transfer of cowpox could be dangerous. One child inoculated by Jenner, a five-year-old boy named John Baker, was never challenged with smallpox. “The boy,” said Jenner, “was rendered unfit for inoculation [with smallpox] from having felt the effects of a contagious fever in a workhouse soon after this experiment was made.” Baker was unfit for inoculation because he had died from a bacterial infection, probably the result of a contaminated cowpox vaccine. In 1861 in Italy forty-one children developed syphilis as a result of arm-to-arm transfer when a small amount of blood from one child in the chain, who had an undiagnosed case of the disease, was injected into others. And in 1883 in Bremen, Germany, arm-to-arm transfer caused a massive outbreak of hepatitis.

Although Edward Jenner made the first viral vaccine, he didn’t know that smallpox and cowpox were related viruses. That was because he’d never heard of viruses. Edward Jenner made his observations several decades before scientists showed what viruses were and how they reproduced.

From Louis Pasteur, a French chemist, Hilleman learned that vaccines could be made from dangerous human viruses. (Jenner had used a cow virus.) Pasteur developed mankind's second vaccine, one that prevented a uniformly fatal disease: rabies.

On July 4, 1885, a rabid dog attacked a nine-year-old boy named Joseph Meister in the town of Meissengott, a small village in the province of Alsace, France. Meister, who was on his way to school, covered his face as the dog knocked him down and bit him fourteen times. A bricklayer walking nearby beat the dog with an iron bar and carried Meister home. The owner later killed the dog and cut open its stomach; out poured straw, hay, and fragments of wood—evidence that the animal had gone mad. (Old stories about infectious diseases often sound like they were written by the Brothers Grimm.)

In ancient times, people with rabies were hunted down like wild animals and strangled or suffocated. By the late 1800s, treatments for rabies had advanced to include the feeding of cock's brains, crayfish eyes, livers from mad dogs, snake skins mixed with wine, and poison from a viper or giving them the "dipping cure," which involved holding victims under water until "they have done the kicking." Techniques that actually worked to prevent rabies included immediately cauterizing a bite with a hot iron or sprinkling gunpowder on the wound and igniting it—processes that killed the virus.

Two days after the attack, Joseph Meister and his mother arrived at the front door of 45 rue d'Ulm in Paris, the home of Pasteur's laboratory. When Pasteur came to the door, Meister's mother dropped to her knees and begged him to save her son. Pasteur took the boy by the hand and gently guided him into his home, later describing the wounded child in his notebook, "Severely bitten on the middle finger of his right hand, on the thighs, and on the leg by the same rabid dog that tore his trousers, threw him down and would have devoured him if it had not been for the arrival of a mason armed with two iron bars who beat down the dog."

For several years preceding Meister's visit to his laboratory, Pasteur had studied rabies virus. To make an experimental rabies vaccine, he found dogs that had died from rabies, ground up their spinal cords, injected infected spinal cords into rabbits, and watched the rabbits die from rabies. Then he removed the rabbits' spinal cords, cut them into thin strips, and dried them in airtight jars. Pasteur found that the longer he dried them, the longer it took for the infected spinal cords to cause disease. After fifteen days of drying, they didn't cause disease at all. Apparently prolonged drying killed rabies virus. Pasteur then performed his groundbreaking experiment. He injected dogs with rabies-infected spinal cords that had been dried for fifteen days and then, successively, with spinal cords that had been dried for fewer and fewer days. At the end of the experiment, Pasteur injected dogs with spinal cords that contained live, deadly rabies virus. Typically the dogs would have died of rabies. But all the dogs that received Pasteur's vaccine survived.

When Joseph Meister came to his laboratory, Pasteur had not yet immunized people, only animals. But at 8:00 p.m. on July 6, 1885, Meister was injected with a rabies-infected rabbit spinal cord that had been dried for fifteen days. Pasteur knew that such a spinal cord didn't kill dogs or rabbits. He could only hope that it didn't kill Meister. During the next eleven days, Meister was injected twelve more times with rabbit spinal cords that had each been less and less dried out and therefore were more and more likely to cause rabies. The final dose, on July 16, was taken from an infected rabbit spinal cord that had been dried for only one day, an injection that would have easily killed a rabbit. Pasteur knew that those final injections were potentially deadly. Writing to his children, he said, "this will be another bad night for your father. [I] cannot come to terms with the idea of applying a measure of last resort to this child. And yet [I have] to go through with it. The little fellow continues to feel very well."

By the end of the month, Meister was home in Alsace, healthy. Using killed, partially killed, and live rabies virus, Pasteur had developed the first vaccine that protected people bitten by rabid animals from developing rabies. Parisians, who had to live every day in fear of rabid dogs prowling their streets, hailed Pasteur's vaccine as one of the greatest medical triumphs of the nineteenth century. But like Jenner's smallpox vaccine, Pasteur's rabies vaccine came with a price. As his vaccine was injected into more and more people, Pasteur found something that he hadn't anticipated: some people—as many as one of every two hundred who used it—became paralyzed and died. At first, Pasteur thought that people were dying from rabies. But they were dying from his vaccine.

Today we understand the problem with Louis Pasteur's rabies vaccine. Cells from the brain and spinal cord contain a substance called myelin basic protein. This protein forms a sheath around nerves like the rubber insulation that surrounds an electrical wire. Some people inoculated with myelin basic protein occasionally develop an immune response against their own nervous systems: autoimmunity. Pasteur's vaccine, made from rabbit spinal cords that contained myelin basic protein, caused autoimmunity. (This, by the way, was why Hilleman cut off the heads of chick embryos before using them. He didn't want to inject children with small amounts of myelin basic protein from the chicks' brains.)

Joseph Meister, who survived the bite of a rabid animal, lived to be sixty years old. When the Nazis occupying Paris in 1940 wanted to see the tomb of Louis Pasteur, Meister, then a guard at the Pasteur Institute, was the first to meet them. But the humiliation of opening his savior's tomb to the Nazi invaders was more than he could handle. Later, locking himself in his small apartment, Meister committed suicide.

From Martinus Beijerinck, a professor of bacteriology at the Delft Polytechnic Institute in the Netherlands, Hilleman learned what viruses were, where they reproduced, and how they caused disease.

As Peter Radetsky describes in *The Invisible Invaders*, Beijerinck “would burst into his lab, a tall, striking figure in a dark coat and high collar. Around the rooms he would prowl, shutting all windows, disdainfully sniffing for the faintest remnant of cigarette smoke, and inspecting benches for as little as a drop of spilled water.” A mean-spirited, haughty, offensive man, Beijerinck often likened his students to untrained monkeys and refused to allow young associates to marry. His personality didn’t limit his achievements, however. In 1898, Martinus Beijerinck performed an experiment that revolutionized microbiology.

Beijerinck was studying tobacco mosaic disease, which stunted the growth of tobacco plants and was common in Europe and Russia. Scientists had already seen bacteria under the microscope, shown that they caused specific diseases, and figured out a method to remove them from water: filtration through unglazed porcelain. (Pots of unglazed porcelain were often kept in the home to purify drinking water.) Beijerinck assumed that bacteria caused tobacco mosaic disease. To prove it, he squeezed diseased plants through a press, collected the sap, rubbed the sap onto healthy leaves, and watched the healthy plants die. Clearly the sap contained the organism that caused the disease. Then Beijerinck performed his seminal experiment. He passed infectious sap through a porcelain filter, and, much to his surprise, found that the sap still caused disease. Beijerinck knew that bacteria should have been trapped by the filter. Something else was getting through.

Beijerinck published his findings in a paper entitled, “Concerning a Contagium Vivum Fluidum as a Cause of the Spot-Disease of Tobacco Leaves.” The term *contagium vivum fluidum* translates as “living contagious fluid.” (Later, Beijerinck referred to the *contagium* as a virus.) Beijerinck said that “the contagium, in order to reproduce, must be incorporated into the living protoplasm of the cell.” Martinus Beijerinck had recognized the single most important difference between bacteria and viruses. Bacteria, capable of independent growth, can multiply on the surface of furniture; in dust; in rainwater; or on the lining of the skin, nose, or throat. But viruses, incapable of independent growth, can reproduce only within the “living protoplasm of the cell.” At the age of forty-seven, Martinus Beijerinck became the father of virology.

Jenner needed cows to make his smallpox vaccine; Pasteur needed dogs and rabbits. From Alexis Carrel, Hilleman learned that animal organs could be kept alive outside of the body, freeing researchers from using whole animals when they wanted to make their vaccines.

On January 17, 1912—three months before the steamship *Titanic* sank in the Atlantic Ocean—Carrel, a French American working at the Rockefeller Institute in New York City, removed a small piece of heart from an unhatched chick embryo and placed it in the bottom of a flask. Every two days he added nutrient fluid that contained chicken plasma and a crude extract made from a chicken embryo. He wanted to see how long he could keep the chicken heart cells alive. Obsessed that the cells might be inadvertently

contaminated with bacteria, Carrel created a cult around its maintenance, insisting that the walls be painted black and that his technicians wear long black gowns with hoods when they entered the room in which it was kept. To celebrate their success, every January doctors and nurses at the Rockefeller Institute lined up outside the research laboratory, locked hands, and joined Carrel in lustily singing happy birthday to the small piece of heart. Carrel and his colleagues maintained the culture of chicken cells until his death in 1944.

From Ernest Goodpasture, who worked in the early 1930s, Hilleman learned that viruses could be grown in eggs, a discovery that forged a permanent bond between virologists and chicken farmers.

Born on a farm near Clarksville, Tennessee, Goodpasture, a quiet, unassuming pathologist, was interested in fowlpox, a virus similar to smallpox. Because fowlpox infected chickens, he decided to try to grow the virus in hens' eggs, reasoning that eggs were sterile (antibiotics hadn't been invented yet) and inexpensive. Working at Vanderbilt University in Nashville, Goodpasture took an incubating hen's egg, bathed it in alcohol, and, to sterilize the shell, set it on fire. Then, using an eggcup as an operating table, he cut a small window in the shell and injected the egg with fowlpox. The virus grew readily in the membrane surrounding the chick embryo; Hilleman used Goodpasture's technique to make his pandemic influenza and mumps vaccines.

From Max Theiler, Hilleman learned that human viruses could be weakened and made into vaccines by growing them in animal cells. (Remember, Hilleman weakened his daughter's mumps virus by growing it in chick cells.)

Theiler, a South African émigré also working at the Rockefeller Institute, wanted to make a vaccine to prevent yellow fever, a tropical viral disease that caused bleeding, (the unmistakable symptom of black vomit), jaundice—a yellowing of the eyes and skin that gave the virus its name—and death. Because yellow fever virus caused severe internal bleeding, it was called a viral hemorrhagic fever. Yellow fever was common in the United States; an outbreak in Philadelphia in the late 1700s killed 10 percent of the city's residents, and an outbreak in New Orleans in the mid-1800s killed 30 percent. The terror once caused by yellow fever is associated today with another viral hemorrhagic fever: Ebola virus.

In the mid-1930s, Max Theiler performed a series of experiments that determined how researchers would make viral vaccines for the next seventy years. Using Carrel's technique of growing chopped-up animal organs in laboratory flasks, Theiler found that yellow fever virus grew in mouse embryos. So he passed the virus from one mouse embryo to another and eventually from one chicken embryo to another. Theiler reasoned that as yellow fever virus got better and better at growing in cells from different species—like mice and chickens—it would become less and less capable of causing disease in man. (Today we know that human viruses forced to grow in animal

cells undergo a series of genetic changes that make them less capable of reproducing and causing disease in people.) To test his theory, Theiler injected a thousand Brazilians with what he hoped was a weakened form of yellow fever virus. He found that most people developed antibodies to the virus and that no one developed the disease. By the end of the 1930s, Theiler had inoculated more than half a million Brazilians, and epidemics of yellow fever in Brazil abated. The yellow fever vaccine made in mouse brains by Max Theiler in the mid-1930s is still used today.

Theiler's technique of weakening human viruses by growing them in cells from other species remains the single most important method to make live weakened viral vaccines. His method has been used to make vaccines against measles, mumps, rubella, chickenpox, and polio. In 1951, "for his discoveries concerning yellow fever and how to contain it," Max Theiler won the Nobel Prize in Medicine. When asked what he planned to do with the \$36,000 in prize money, he said "Buy a case of Scotch and watch the [Brooklyn] Dodgers."

Like Jenner and Pasteur before him, Theiler also saw tragedy follow his vaccine. In the early 1940s, scientists made Theiler's vaccine using human serum obtained from several volunteers, to stabilize the virus. Unfortunately, unnoticed at the time, at least one of these volunteers was jaundiced, infected with hepatitis B virus. As a consequence, more than three hundred thousand American servicemen injected with contaminated yellow fever vaccine developed hepatitis, and sixty died. Human serum was never again used to stabilize vaccines.

From the research team of John Enders, Thomas Weller, and Frederick Robbins, working at Boston Children's Hospital (part of Harvard's Medical School) in the late-1940s, Hilleman learned how to grow animal and human cells in the laboratory. Alexis Carrel's technique, using chopped up animal organs, was called tissue culture; the Enders group's technique, using single layers of animal or human cells grown in laboratory flasks, was called cell culture. Now when researchers want to grow viruses, they simply take a vial of cells out of the freezer, thaw them out, place them into laboratory flasks, watch them reproduce until a single layer neatly covers the bottom of the flask, and inoculate them with viruses. The days of growing viruses in whole animals or chopped-up animal organs were over. The Enders group's technique is still used to make viral vaccines today.

One of the Boston group's first cell cultures was made from a human fetus. On March 30, 1948, at 8:30 a.m., Thomas Weller walked across the street in front of Boston Children's Hospital and into the office of Duncan Reid, an obstetrician working at the Boston Lying-In Hospital who had just aborted a twelve-week pregnancy; the mother had chosen to end her pregnancy because she had been infected with rubella, a virus known to cause birth defects. Reid handed Weller the fetus. After coaxing fetal cells to reproduce on the bottom of laboratory flasks, Weller found that polio virus grew in the cells. Later, Weller, Robbins, and Enders found that polio virus also grew in a variety of

different animal and human cells. Prior to these experiments, polio virus could only be grown in cells from brains and spinal cords; researchers feared using a polio vaccine made from nervous tissue for the same reason that they feared Pasteur's vaccine, the dangerous side effect of autoimmunity.

In 1954 Enders, Weller, and Robbins won the Nobel Prize in Medicine for "their discovery of the ability of polio viruses to grow in cultures of various types of tissue." These studies allowed Jonas Salk and Albert Sabin to make polio vaccines that eventually eliminated polio from most of the world.

From Jonas Salk, the scientist who first found a way to prevent polio, Hilleman learned that vaccines could win the heart of the American public.

Born and raised in New York City, the son of Russian immigrants, Salk was driven, obstinate, and self-assured. Working at the University of Pittsburgh in the early 1950s, Salk used the Enders group's technique to grow poliovirus in monkey kidney cells. Then he purified the virus, killed it with formaldehyde, and injected it into seven hundred children in and around Pittsburgh. Salk reasoned that killed poliovirus would induce polio antibodies but wouldn't cause polio. In 1954, funded by the March of Dimes, doctors and nurses injected four hundred thousand children with Salk's vaccine and two hundred thousand with an inert liquid that looked like vaccine, called placebo. The program was then and remains today the largest test of a medical product ever performed. Following the announcement that the vaccine worked, Americans named hospitals, schools, streets, and babies after Salk and sent him money, clothes, and cars. Universities offered him honorary degrees and countries issued proclamations in his honor. Salk started the day as a scientist at the University of Pittsburgh and ended it as one of the most revered men on the face of the earth. When people hear the word *vaccine* today, the first person they think of is Jonas Salk.

But like Jenner, Pasteur, and Theiler before him, Salk watched tragedy follow his vaccine. When Salk found that polio vaccine could be made by inactivating polio virus with formaldehyde, five companies stepped forward to make it; on April 12, 1955, each of those companies were permitted to sell their vaccine to the public. One company, Cutter Laboratories of Berkeley, California, made it badly. Researchers and executives at Cutter were confident that they had made their polio vaccine exactly as Jonas Salk had proscribed, giving it to the children of four hundred and fifty of their employees. But, because Cutter researchers hadn't properly filtered out the cells in which they grew poliovirus, some virus particles effectively escaped the killing effects of formaldehyde. As a consequence, more than one hundred thousand children were inadvertently injected with live, dangerous poliovirus. Worse, children injected with Cutter's vaccine spread polio to others, starting the first and only man-made polio epidemic. When the dust settled, live poliovirus contained in Cutter's vaccine had infected two hundred thousand people; caused about seventy thousand to develop mild polio; permanently and severely paralyzed two hundred people, mostly children; and killed ten. It was one



of the worst biological disasters in American history. Federal regulators quickly identified the problem with Cutter’s vaccine and established better standards for vaccine manufacture and safety testing. Cutter Laboratories never made another polio vaccine again. And Salk’s polio vaccine helped to dramatically reduce—and in some countries eliminate—one of the world’s most crippling infections.

Hilleman had learned from what had gone right and what had gone wrong before him. By the time he made his mumps vaccine, an enormous path had been cleared through the thicket. “It was an age of genius,” he said. “I was able to do what I did because of what they did.”