A Short History of Vaccination

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Vaccination as a deliberate attempt to protect humans against disease has a short history when measured against the thou- sands of years that humans have sought to rid themselves of plagues and pestilence. Only in the 20th century did the prac- tice flower into the routine vaccination of large populations. Yet, despite its relative youth, the impact of vaccination on the health of the world’s peoples is hard to exaggerate. With the exception of safe water, no other intervention, not even anti- biotics, has had such a major effect on mortality reduction and population growth.

**SECTION**

**1 General Aspects of Vaccination**

Since the first vaccine was introduced by Edward Jenner ([Fig. 1.1](#_bookmark0)) in 1798, vaccination has controlled 14 major dis- eases, at least in parts of the world: smallpox, diphtheria, tetanus, yellow fever, pertussis, *Haemophilus influenzae* type b disease, poliomyelitis, measles, mumps, rubella, typhoid, rabies, rotavirus, and hepatitis B. For smallpox, the dream of eradication has been fulfilled; naturally occurring smallpox has disappeared from the world.1 Cases of poliomyelitis have been reduced by 99% and this disease also is targeted for eradication. Rubella and congenital rubella syndrome have been officially declared eliminated from the Americas as of 2015.2 Vaccinations against many other diseases have made major headway. The path to these successes is worth examining.3–5

# EARLY DEVELOPMENTS

Attempts to “vaccinate” began long before Edward Jenner’s smallpox vaccination. While the precise origin of **variolation** remains unknown, it seems to have developed somewhere in Central Asia in the early part of the second millennium and then spread east to China and west to Turkey, Africa, and finally Europe.

In the 7th century, some Indian Buddhists drank snake venom in an attempt to become immune to its effect. They may have been inducing antitoxin-like immunity.6 In the 16th century, Brahmin Hindus in India practiced a form of variola- tion by introducing dried pus from smallpox pustules into the skin of a patient.7 Writings that cite the use of inoculation and variolation in 10th-century China8–10 make interesting reading but apparently cannot be verified.11 There is, however, 18th-century documentation of Chinese variolation. *The Golden Mirror of Medicine*, a medical text dated 1742, listed four forms of inoculation against smallpox practiced in China since 1695:

* The nose plugged with powdered scabs laid on cotton wool
* Powdered scabs blown into the nose
* The undergarments of an infected child put on a healthy child for several days
* A piece of cotton smeared with the contents of a vesicle and stuffed into the nose8,11

This text, endorsed by the Imperial Court, raised the status of variolation in China, which previously had been considered just a folk remedy. Another Chinese text, published a century before Jenner’s work, stated that white cow fleas were used for smallpox prevention.9 The fleas were ground into powder and made into pills.

**Variolation** was introduced into England by Lady Mary Wortley Montagu in 1721, after her return from Constanti- nople, where she lived for 2 years with her husband, the British Ambassador to the Ottoman Empire. Lady Montagu had been disfigured by smallpox earlier in life and her 20-year- old brother had died of the disease. While living in Turkey, she frequently observed variolation and wrote to a friend back home: “The small-pox, so fatal, and so general amongst us, is here entirely harmless, by the invention of engrafting, which is the term they give it…. Every year, thousands undergo this operation…they take the small-pox here by way of diversion, as they take the waters in other countries. There is no example of any one that has died in it….”12 So impressed was she that she had her own son variolated while still living in Turkey. Dr. Charles Maitland, who performed the procedure on her son in Constantinople, later performed the first variolation in England in 1721 on Lady Montagu’s daughter. The treatment was effective, but results were erratic, and 2% to 3% of persons treated died of smallpox contracted from the variolation itself.13

The English medical community had previously learned of

variolation in 1713 when Emanuel Timoni, MD, a graduate of Oxford University living in Turkey, sent a letter to the Royal Society about variolation. Another physician, Giacomo Pilar- ino, also reported Turkish variolation to the Royal Society in 1716.14 The reports did not seem to be significant, and the procedure was not adopted.15 An earlier hint of variolation is made by the Danish physician Thomas Bartholin of Copen- hagen in 1675, who mentioned that there was a “market” in Copenhagen where people would go to buy the poxvirus from enterprising housewives. It is unclear whether these purchases were for the prevention of smallpox in healthy persons or for the treatment of persons already infected.15–19 Voltaire lauded the variolation of Circassian women to maintain their beauty in his *Lettres Philosophiques* in 1721.

Most intriguing, at the same time that Charles Maitland variolated Lady Montagu’s daughter in England (1721), vari- olation was practiced in America at the instigation of Cotton Mather who first learned of it from his African slave, Onesi- mus.15 Mather subsequently read about variolation in volume 29 of the *Philosophical Transactions of the Royal Society of London*, 1714 to 1716, which contained the aforementioned articles by Timoni and Pilarino. There was a smallpox epidemic in Boston at that time, and Mather used the authority and pres- tige of his position to urge all Boston-area physicians to con- sider the practice of variolation (letter, June 24,1721). Physician

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**Figure 1.1.** Edward Jenner. *(Courtesy the Institute of the History of Medicine, The Johns Hopkins University, Baltimore, MD.)*

response was negative, except for Dr. Zabdiel Boylston, who successfully inoculated his own 6-year-old son and two black slaves shortly thereafter. Six weeks later, Dr. Boylston vario- lated Mather’s son, Sammy, and publicized its success. Mather continued to harangue recalcitrant physicians and the public about variolation to the point where a grenade was thrown into his house in utter exasperation!20,21

Despite the known risks, George Washington was com- pelled in the 1770s to order that Continental Army recruits undergo variolation against smallpox, to which the Americans were highly susceptible; the great majority of their English enemies were immune from early childhood exposure or from variolation.22 In the mid-18th century, several treatises were written on inoculation against measles as well; the Scottish physician Francis Home successfully inoculated humans against measles and published his results (1758).23–25

In 1774 in Yetminster, England (Dorset County), a cattle breeder named Benjamin Jesty, himself immune to smallpox after contracting cowpox from his herd, deliberately inocu- lated his wife and two children with cowpox to avoid a small- pox epidemic.14 This was no spur-of-the-moment idea; like many country farmers in the area, he knew that dairymaids seemed to be protected from smallpox after they had con- tracted cowpox. He had considered the possibility of deliber- ately using the inoculation technique with cowpox for quite some time and acted only when there was an imminent threat of a smallpox outbreak. He took his wife and two children to a nearby field where he knew he could find cattle with cowpox. He inoculated all three of them. His experiment succeeded; they were unaffected by the outbreak, and his two sons were still immune 15 years later, when they were deliberately vari- olated with smallpox.26–28

Jesty’s story is interesting; although neither a physician nor

a scientist, he nevertheless reflected on the evidence of local

dairymaids’ immunity to smallpox because of prior infection with cowpox and saw the principle involved: inoculation with one moderately harmless disease (cowpox) could provide pro- tection against another far more dangerous disease, smallpox. When Jesty’s neighbors learned that his wife had developed inflammation at the site of the inoculation and had to be treated by a physician because he had “vaccinated” her, they vehemently scorned him.28 Jesty retreated in the face of their disapproval and never attempted to publicize his experiment or to vaccinate anyone else.27 Truly, Jesty’s actions constituted the first known real **vaccination**—the use of cowpox to protect against smallpox.

Some 30 years later, after Jenner had popularized vaccina- tion, and thanks to the intercession of an enthusiastic vaccina- tor named Rev. Andrew Bell, Jesty was invited to London in 1805 by the Original Vaccine Pock Institute to tell the story of his 1774 “experiment” before the Institute’s examiners. At the end of the visit, they issued a public statement in the *Edinburgh Medical & Surgical Journal* recognizing Jesty’s cowpox vaccina- tion. They commissioned his portrait as well, which was hung in the Institute.27,28 This was a vindication of sorts, but he did not share in the significant monetary award that Jenner received. When Jesty died in 1816, his wife made certain that his tombstone recorded for all posterity his central role in the great endeavor (see frontispiece).

Despite Jesty’s successful vaccination of his family, Jenner’s work with cowpox vaccination still holds title to the first sci- entific attempt to control an infectious disease on a large scale by means other than transmitting the disease itself.29

Cowpox was not a widespread infection. It appeared spo- radically in certain rural counties of England. Thus, the local wisdom that persons who contracted cowpox “did not take the smallpox” was not widely known. Jenner knew it because he had been an apothecary apprentice in Chipping Sodbury in 1768, where a milkmaid told him about using cowpox against smallpox. Indeed, he discussed the possible associa- tion between the two with John Hunter, with whom he studied in London from 1770 to 1773. For unknown reasons, Jenner did not return to the subject of cowpox/smallpox until 1796. His first manuscript to the Royal Society on vaccination was rejected because his experiment involved only one person, not enough to establish a principle.20,27 Within 2 years, he expanded his studies and proved that cowpox could be passed directly from one person to another, thereby providing “large-scale” inoculation against smallpox without depending on the spo- radic outbreaks of natural cowpox. Jenner self-published his results in *Variolae Vaccinae* in 1798.29 This publication brought to the attention of the entire medical community the merits of inoculation with the relatively obscure animal disease, cowpox, to prevent one of humankind’s deadliest scourges. Fortunately for the world, Jenner had the connections that Jesty did not, and vaccination rapidly replaced variolation. While Jenner did use the term “vaccine,” he is not the origina- tor of the term *vaccination*; that honor belongs to his friend Richard Dunning, who used it in 1800.30,31 By 1810, Jenner realized that immunity against smallpox by vaccination was not lifelong, but he did not know why.13

Curiously, the vaccinia virus used in current smallpox

vaccine is not the cowpox virus that Jenner used. Vaccinia, cowpox, and variola are all related orthopox viruses, suspected to have been derived from a common ancestor.32 S. Monkton Copeman33–35 gave considerable attention to this issue in the Milroy Lectures of 1898, which make fascinating reading. He documents the many ways, times, and methods by which cows were inoculated to keep the supply of cowpox intact, further adding to the confusion of when and how vaccinia replaced cowpox.33–35 It has been suggested that vaccinia may have originated in a now extinct horsepox.36 What is clear is that

the exact origin of vaccinia remains unknown, as does how it became substituted for cowpox.

For the first several decades of the 19th century, arm-to-arm transfer was the primary method of human vaccination.37 Other recognized diseases, such as syphilis and tuberculosis, were known to be occasionally transmitted along with the “cowpox” virus, so a search was underway to find an alterna- tive way to vaccinate and to ensure a steady supply of cowpox vaccine. The concept of “passages” of the immunizing agent (transmission from one human or animal to another) was well known, and in 1836, Edward Ballard38 argued for choos- ing new strains of cowpox for vaccination because the old strains were too weak (too attenuated) from so many arm-to- arm passages. He recommended that the lymph (vesicle fluid) be passed back through a calf to regain strength.

According to Ballard, the idea to use animals to propagate cowpox vaccine, as opposed to human arm-to-arm propaga- tion, was first practiced in Naples, Italy, in 1805. Troja used vaccine virus derived from humans to inoculate cows and then used the lymph from the cows’ pocks to vaccinate humans. This was referred to as *retrovaccination*. Troja’s successor, Gal- biati, stated that he used bovine lymph specifically to avoid transmission of other human diseases. By 1842, a third Italian, Negri, gave up the practice of retrovaccination entirely. He started what was called *animal vaccination,* that is, inoculating from one cow to another cow to keep a steady supply of cowpox lymph. But the initial virus source that he inoculated into the cow was from a human! When a natural outbreak of cowpox occurred in Calabria, Negri switched to that source for his lymph, but because he was running a commercial activity, he bought a third “cowpox strain” from London. Its origin was questionable, but Negri used it anyway, from 1858 onward. By mid- century, arm-to-arm vaccination was essen- tially replaced by animal vaccination but it is easy to under- stand why the origin of the vaccinia virus is difficult to trace.35,38 In 1864, a French physician named Lanoix studied animal vaccination in Naples and brought back to France a calf inocu- lated by Negri. He and Chambon set up a business in France for production of calf-to-calf lymph vaccine for humans.15,35,38 Subsequently, the French government became interested and ordered a study of animal vaccination. The lymph used in the experiments was obtained from Negri in Italy, but was really from the London cowpox lymph of questionable origin. By chance, there were two outbreaks of cowpox in France in 1866. Lanoix and Chambon collected cowpox from both, mixed them together, and used the mixture to produce their vaccine.35,38 From these confused beginnings, animal vaccina-

tion spread rapidly throughout the continent.

Following Robert Koch’s recommendation, German scien- tists began to use glycerin to kill bacteria and to preserve the lymph.39 This generated a ready supply of a stable calf lymph of consistent potency.13 By the end of the 1890s, the use of glycerinated calves’ lymph was standard everywhere, and both arm-to-arm vaccination and unglycerinated animal vaccina- tion were abandoned.

# Louis Pasteur and the Age of Vaccination

Louis Pasteur’s ([Fig. 1.2](#_bookmark1)) work on the attenuation of the chicken cholera bacterium in the late 1870s was the first major advance after Jenner’s *Variolae Vaccinae*. Pasteur drew on con- cepts that had been developing for at least 40 years: attenua- tion; modification through passage; renewed virulence; and, most important, the need to replace person-to-person (or animal-to-animal) vaccination with something safer, consis- tent, and less likely to transmit other diseases.40

The popular story that Pasteur had a “eureka” moment when he noticed that a chicken cholera culture (*Pasteurella*



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**Figure 1.2.** Louis Pasteur. *(Courtesy the Pasteur Institute, Paris.)*

*multocida*) left exposed to air during a long holiday period provided immunity when challenged is considerably more complex than originally told. From the moment he obtained the chicken cholera culture from Henry Toussaint in October 1878, Pasteur spoke of wanting to manipulate it to make a vaccine. The culture was virulent, and he killed many chickens trying to keep it alive through passage from chicken to chicken. By January 1879, he found that he could keep the microbe alive in a culture of chicken bouillon. Chickens inoculated from this bouillon culture died, so it had retained the viru- lence of his original sample. Next, he prepared a bouillon culture using inflamed chicken muscle tissue from the site of inoculation. He noted that this infected muscle tissue culture did not develop normally as the other cultures did; it became acidic. He fed the chickens bread soaked in the first bouillon culture and then fed them bread soaked in the muscle bouil- lon. They became sick but survived. He then challenged them with virulent organisms and they lived, so he thought he had a vaccine. After the challenge, the chickens sickened again but again survived and eventually returned to health. So by March 1879, Pasteur realized that he had attained “resistance” to disease; he also knew he had not yet found a product that could be safely used as a vaccine. For the next several months, Pasteur subjected the chicken cholera microbe to various con- ditions, for example, in a vacuum, exposed to air, and various intervals before inoculation. But it was mainly the chickens that had eaten the bread soaked in acidic bouillon that survived.

Pasteur left on vacation at the end of July 1879; he returned

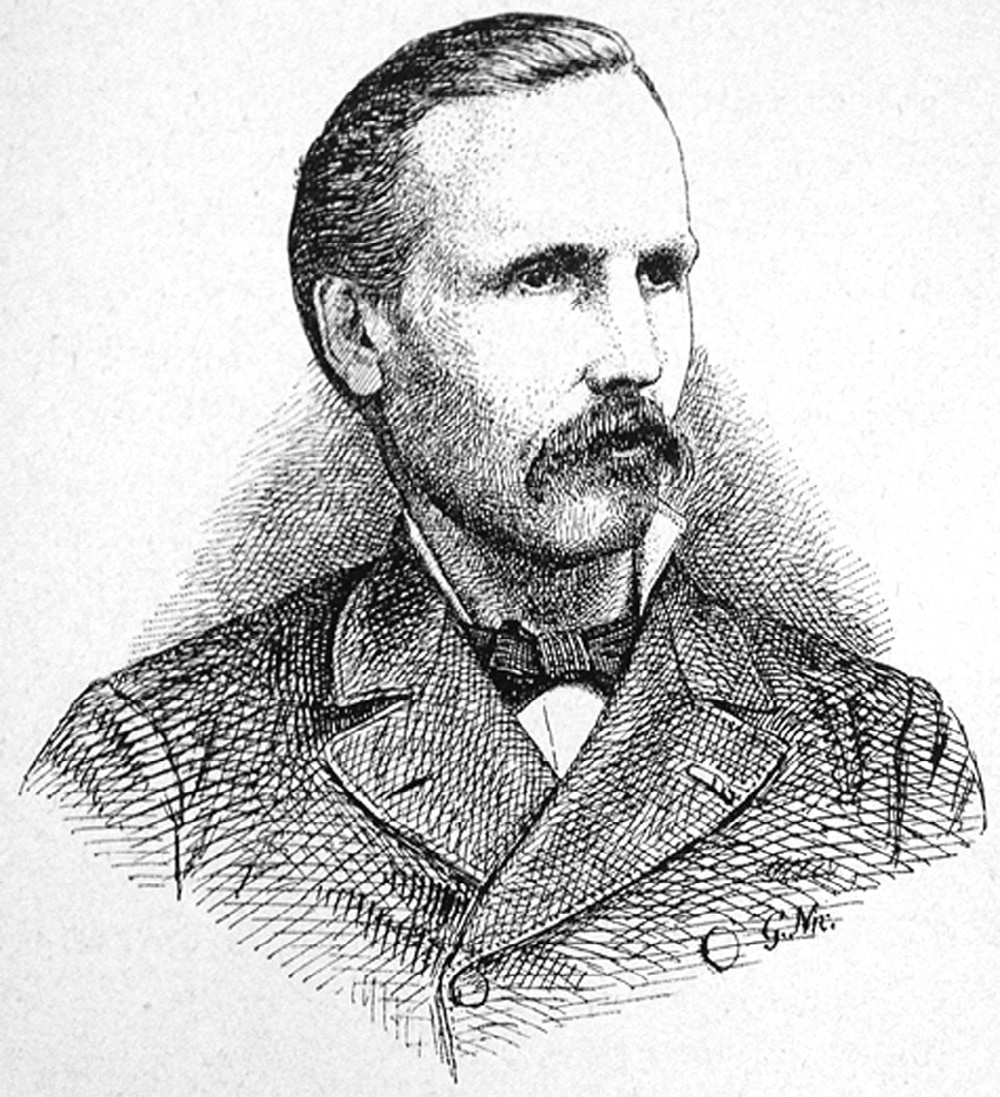
in October and again took up his chicken cholera experiments, focusing on the cultures that had become acidic because they seemed to be the ones that conferred immunity. He tinkered with the amount of time the microbe remained in the acidic cultures. On December 18, 1879, he noted that two cultures made from a chicken that died of a pre-vacation inoculation were the only cultures that turned acidic. He focused on them for his vaccine, although he still had many problems with illness in the chickens. By the middle of January 1880, Pasteur realized that the diminution of virulence came from leaving the microbe for a long time in an acid culture.

Pasteur’s definitive test was on January 22, 1880. With highly virulent chicken cholera, he inoculated 19 naïve chick- ens and 8 that had been previously immunized twice with his acidic cultures. All eight of the previously immunized chickens lived. Most of the naïve candidates did not. So Pasteur’s eureka moment was really the result of many months of intensive, deliberate research. Pasteur presented his results at the Acadé- mie des Sciences on February 8, 1880, and at the Académie de Médicine on February 10, 1880. He announced that he had achieved successful vaccination against chicken cholera, but he did not reveal his technique. He continued to refine his technique and tried to increase the stability of the final product. Finally, in October 1880, he published at least part of his method for preparation of the vaccine.20,41 Pasteur’s chicken cholera vaccine harkened back to the classic variola- tion technique, which had used a weakened form of smallpox to inoculate against smallpox. Therefore, the modern concept of vaccination, involving the development of vaccines in the laboratory and using the same agent that caused the disease, was truly introduced with Pasteur’s chicken cholera vaccine, 5 years before the famous vaccination of Joseph Meister against rabies. Ironically, the chicken cholera vaccine was never a success; there were frequent vaccine failures. Pasteur was lauded, but the vaccine was eventually discontinued.20

Pasteur’s research on anthrax began in 1877 and over-

lapped his work on chicken cholera. Casimir Davaine had seen the anthrax bacillus in 1850, and had postulated it as the cause of anthrax,42,43 but Koch was the first to obtain pure cultures of anthrax bacillus and to describe its capability to survive indefinitely in the form of spores.44 He transmitted it to several laboratory animals and proved that there was a causal relationship between this bacillus and the disease anthrax.

Pasteur knew of Davaine’s and Koch’s work and that of the veterinarian Toussaint ([Fig. 1.3](#_bookmark2)).45 Indeed, he was in a neck- and-neck competition with Toussaint to develop an anthrax vaccine. Toussaint published two articles in July 1880 in the *Comptes Rendus de l’Académie des Sciences* on a live anthrax



**Figure 1.3.** Henry Toussaint.

vaccine that he developed and tested, which induced immu- nity against anthrax.20,46–48 He had started his anthrax experi- ments in May 1880, stating that he was inspired to do so after hearing Pasteur in February 1880 describe his chicken cholera vaccine experiment, although Pasteur had not then revealed his method. Toussaint used the blood of bovines that had died of anthrax, noting that the fluid was full of bacteria, which others thought was extraneous material but Toussaint thought was the causative agent of anthrax. He first attempted to simply “filter” the blood and use it as a vaccine but quickly realized that the bacteria could pass through the filter. He then decided to follow the procedure that Davaine described: to heat the blood for 10 minutes at 55°C or subject the blood to the action of diluted phenol. He used both of these techniques—and a variation on filtering in which he used 12 filters—to produce an attenuated vaccine that he injected in rabbits, sheep, and young dogs. The animals were protected by all three methods, although Toussaint initially thought that the heat-treated was the best.

Toussaint’s articles caused quite a stir at the Académie;

Pasteur and others challenged their validity and Toussaint was compelled to not only reveal his methods, but also to conduct experiments to prove his claim. On July 28, while still in Toulouse, he took fresh blood from a sheep dying of anthrax and prepared his vaccine in two lots, one with 1% phenol and the other with 1.5%. Both lots were filtered, although by dif- ferent means. *Neither lot was heat-treated*. He left for Paris with his two lots of “vaccine,” where his experiment continued at Vincennes and Alfort under the watchful eyes of several researchers. On August 8, Toussaint inoculated 20 sheep with the first solution (1% and filter papers); 4 of them died very quickly, but the other 16 survived. On August 22, six new sheep were inoculated with material from the second lot (1.5% phenol and rudimentary filtration), and all survived without illness. All 22 sheep were then subjected to challenge with virulent anthrax and all survived. With one injection of a (partially) live vaccine attenuated by filtration and phenol acid, Toussaint had achieved immunity to anthrax!20

It should be reemphasized that Pasteur did not reveal the

method for making chicken cholera vaccine until October 1880. Toussaint’s vaccine work was original; it was his own, not Pasteur’s. He indeed induced immunity to anthrax, and his was the first anthrax vaccine. Eventually, he received the Prix Vaillant and the Légion d’Honneur for this work.20,46–49 Toussaint came down with a debilitating neurologic disease in 1881, which prevented him from pursuing his claim as the originator of the first anthrax vaccine. His health continued to decline, and he died in 1890 at age 43 years.20,46

The following spring, Pasteur announced the first *public* controlled experiment of anthrax vaccination at Pouilly-le- Fort on May 5,1881.50 It was initiated by Pasteur in an effort to silence his many critics and to gain recognition for his own anthrax vaccine. Pasteur inoculated 24 sheep, 1 goat, and 6 cows with attenuated anthrax bacilli. On May 17, these same animals were inoculated again with more virulent but still attenuated anthrax bacilli. At the same time, 24 sheep, 1 goat, and 4 cows were kept as control animals and given no inocula- tions. On May 31, both groups were inoculated with virulent anthrax from spores that Pasteur had kept in his laboratory since 1877.

By the 2nd of June, 21 of the nonvaccinated sheep and the nonvaccinated goat were dead. Two more nonvaccinated sheep died before the spectators’ eyes, and the last one died before day’s end. All vaccinated sheep, the vaccinated goat, and the six cows remained healthy. (The nonvaccinated cows did not die but showed clear evidence of having contracted anthrax. Their size perhaps had saved them.) At the end of this experiment, the triumphant Pasteur wrote that he had shown

that vaccines could be made that were cultivatable at will by a method that could be generalized, since he had already used the method previously to create a vaccine against chicken cholera. His experiment represented a considerable advance- ment over Jennerian vaccination, which had not been sub- jected to the rigors of a controlled experiment.

It has since been documented that Pasteur’s results with chicken cholera and anthrax were not as clear-cut as previously thought. Pasteur deliberately withheld critical data (conflict- ing information on the degree of protection of the vaccine) in his communications to the Académie de Médicine.51–53 However, this in no way detracts from the significance of his findings, which proved that one could “create” standardized, reproducible vaccines. Pasteur’s experiments with chicken cholera and anthrax41,50 announced to the world that a new, scientific era in vaccination had begun.

By the time the **rabies** vaccine was first administered to humans in 1885,54 the general public and the scientific com- munity were well aware of the “new vaccination,” but only in relation to animals. The reaction when Joseph Meister and Jean Baptiste Jupille became the first humans to be vaccinated against rabies was predictable: outrage. Meister, a 9-year-old boy from Alsace, had been bitten 14 times on the hands and thighs and arrived in Paris some 60 hours after he had been attacked by a rabid dog. The physician working with Pasteur, Dr. Joseph Grancher, was convinced that Meister would die of rabies if left untreated and, therefore, the attempt to vaccinate was justified to save his life. Meister was vaccinated in the same manner that Pasteur was using in his experiments to protect animals (especially dogs) against rabies: with a series of pro- gressively less dried and, therefore, more virulent rabbit spinal cords obtained from rabbits that had died of rabies after having received “fixed virus” injections of rabies virus. A couple of months later, Jean Baptiste Jupille, a 14-year-old from the Jura region of France, arrived 6 days after having been bitten multiple times. (He had fought off a rabid dog that had attacked a number of younger children.) He was given the same course of treatment that Meister received. They both survived.13

That Pasteur had deliberately introduced a deadly agent

into a human left people aghast. The fact that the rabies virus had been attenuated did not appease the general public or many in the medical community; the cases of rabies that occasionally occurred in subsequent vaccinees were attributed to the vaccine and were viewed as medical murders. Even Émile Roux, one of Pasteur’s staunchest allies and a collabora- tor in the rabies experiments, was appalled at the vaccination of Meister. He thought it was unjustified by the experiments conducted up to that point.

An examination of Pasteur’s laboratory notebooks indi- cates that Roux was right to object.52 The notebooks tell us that, shortly before vaccinating Meister, in May and June of that same year, Pasteur had seen and recommended vaccina- tion for two other people in local hospitals, each of whom had been admitted with the presumed diagnosis of rabies. The first, an adult, was admitted with an uncertain diagnosis of rabies. The second case was a young girl, 11 years old, who had been bitten by a rabid dog on the lip, remained untreated, and was admitted to a hospital a month later with frank rabies. In both cases, Pasteur used a rabies vaccine made of an emulsion of dessicated spinal cord from a rabid rabbit. Up to this point, he had never published anything about using spinal cords as a vaccine, and in fact, had not yet successfully protected any animal from rabies with such a vaccine. The first patient, the adult whose diagnosis was uncertain, received only one–unauthorized–dose of the vaccine before his doctor forbade the administration of further doses. This patient lived. The young girl, whose rabies diagnosis was not in doubt,

received two injections of the same vaccine on her first day in the hospital. When Pasteur and his nephew Adrian Loir arrived the next morning to give the third injection, the young girl died before she could receive it.52 The death was almost cer- tainly from rabies and not from the two injections she had received. These two cases were never published by Pasteur but were subsequently found in his laboratory notebooks.

Roux left Pasteur’s laboratory in protest after Meister’s vac- cination and did not return until the summer of 1886, after several dozen people who had been bitten by rabid animals had been successfully vaccinated.13,52,55,56 Ultimately, hundreds were saved from rabies, many more than had died despite the vaccination (presumably from rabies contracted from the bites). Unfortunately, that did not lessen the strenuous oppo- sition to rabies vaccination in humans nor the belief by many that the vaccination itself caused the deaths. After all, only 45 years earlier, once Jenner’s vaccination had been accepted, variolation had been made a felony in England for the very same reason: it introduced a deadly live virus into humans (as opposed to cowpox, which was not deadly).13 Grancher, the physician who administered the rabies vaccine to Meister and many others, was one of Pasteur’s staunchest supporters and was invaluable in defending Pasteur before the Académie de Médicine and against recalcitrant physicians.56 Despite the opposition, and thanks to Grancher and other supporters, Pasteur soon became a worldwide medical hero.

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The next major step in vaccine development took place in

the United States. It involved a new concept that was equally important: killed vaccines. In 1886, Daniel Elmer Salmon ([Fig. 1.4](#_bookmark3)) and Theobald Smith ([Fig. 1.5](#_bookmark4)) published their work on a killed hog cholera “virus” vaccine.57,58 The heated suspen- sion of organisms immunized pigeons against the disease. The vaccine they developed was actually a bacterial vaccine against



**Figure 1.4.** Daniel Elmer Salmon. *(Courtesy the American Veterinary Medical Association, Gallery of AVMA Presidents.)*



**Figure 1.5.** Theobald Smith. *(From Cohen B, ed. Chronicles of the Society of American Bacteriologists, 1899–1950. Washington, DC: American Society for Microbiology; 1950:36.)*

a cholera-like salmonellosis,59 but the term *virus* in the latter half of the 19th century did not have the specific meaning it has today; there was confusion about what pathogens could pass through filters. Their report demonstrated that the ideas of **live and killed vaccines** developed almost simultaneously. This seminal work of Salmon and Smith bore fruit for *humans* 15 years later.

Ironically, their competitors in the development of a killed vaccine were Charles Chamberland and Roux from Pasteur’s laboratory, who reported on the same topic in December 1887,60 some 16 months after Salmon and Smith’s original paper. In 1888, Salmon read a paper before the American Association for the Advancement of Science (AAAS) defending their 1886 article and their priority in developing the first killed vaccine.61 However, the Institut Pasteur had just been established in 1887; Pasteur was at the height of his fame and worldwide prestige thanks to the rabies vaccine. Not surpris- ingly, Salmon and Smith, working for the U.S. Department of Agriculture, saw their claim lost in the aura surrounding Pasteur and his associates. Thus, even 100 years ago, the Insti- tut Pasteur and the U.S. government were involved in disputa- tions about discovery rights, similar to the late 20th-century controversy about who first isolated the human immune defi- ciency virus: Luc Montagnier at the Institut Pasteur or Robert Gallo at the National Institutes of Health.

Killed vaccines for typhoid, plague, and cholera followed

on the heels of Salmon and Smith’s research. Richard Pfeiffer and Wilhelm Kolle in Germany and Almroth Wright in England worked independently on killed **typhoid vaccines.**62–65 To this day, the debate continues about exactly who inoculated the first human with killed typhoid vaccine. In truth, all three deserve credit because it is now clear that several groups were working on typhoid vaccine at that time.66

Shibasaburo Kitasato and Alexandre Yersin, each working independently, discovered the causative bacillus of the **plague** in 1894, *Yersinia pestis* (called *Pasteurella pestis* until 1970).13,15,67,68 With Albert Calmette and Amédée Borelle, Yersin developed a killed plague vaccine for animals,69 but it was Waldemar Haffkine who took up the task of developing a vaccine against human plague.70,71 Haffkine was in India working on cholera vaccine when bubonic plague broke out in Bombay. He switched to studies of plague immunization and was himself the first to be injected with his new killed plague vaccine. More than 8000 people were then vaccinated within a few weeks. For a while, Haffkine was a hero. However, the Mulkowal incident in 1902 when 19 people died from contaminated plague vaccine resulted in Haffkine’s removal from his post by the Indian government. The contamination (with tetanus bacillus) does not seem to have been his fault.15 Nevertheless, his scientific career and reputation were severely damaged; he never fully recovered from the incident and retired early from science at age 55 years. Later, with the wisdom of hindsight, the Indian government renamed the Plague Research Laboratory where he had worked *The Haffkine Institute*. Perhaps as important as his development of the plague vaccine was Haffkine’s contribution to the literature on the proper way to conduct controlled field trials.72

John Snow had shown that cholera was transmitted by

contaminated water in 1848,73 although he did not know the identity of the contaminant. That answer was supplied by Koch, when he isolated *Vibrio cholerae* as the causal organism in 1883.74 Early attempts at a **cholera** vaccine were made by Jaime Ferrán, Pasteur’s pupil, and by Haffkine. Both used live cultures and both vaccines were rejected because of severe reactions.13 Kolle developed a heat-killed, human cholera vaccine in 1896.75,76 He grew the vibrios in agar, suspended them in saline solution, heated them at 50°C for a few minutes (later changed to 56°C for 1 hour), and then added 0.5% phenol.

In parallel with the focused research on vaccines, important work on immunity was being pursued at the end of the 19th century. Elie Metchnikoff, another Pasteur protégé, reported his theory of cellular immunity in 1884.13,77 He named the cells that ingested and destroyed invading microorganisms and other foreign bodies *phagocytes*. Although he did not understand the role of serum and plasma in immunity at this early date, his work was truly pioneering.

In 1888, Roux and Yersin showed that the **diphtheria** bacil- lus produced a powerful **toxin**.13,78 Two years later, Emil von Behring and Kitasato, working in Koch’s laboratory in Berlin, followed up on early work by Karl Fraenkel; they showed the presence of powerful **antitoxins** in the serum of animals previously injected with low doses of **tetanus** or diphtheria toxins.79–81 The antitoxin neutralized diphtheria or tetanus toxin in culture. Further experiments showed that the anti- toxin protected animals challenged with the tetanus or diph- theria bacillus. Although he did not use the term, what von Behring had found in the serum of animals previously injected with diphtheria or tetanus toxins were **antibodies**. It was Paul Ehrlich, also working in Koch’s laboratory, who first referred to these antitoxins as antibodies—“antikorps.”82

Progress occurred rapidly after these reports; the first child was treated with diphtheria antitoxin just 1 year later, Decem- ber 1891. Shortly thereafter, commercial production of diph- theria antitoxin began. von Behring referred to the rabbit serum that contained the antitoxin as “immune serum.” Soon, the process of inoculating with the immune serum that con- tained tetanus or diphtheria antitoxin was referred to for the first time as **immunization**.15,81

Ehrlich’s receptor theory of immunity, which he referred to as the “side-chain theory,” made a strong contribution to

vaccine development. When it was first developed in 1897, the theory was used primarily to explain toxin–antitoxin interac- tions and subsequently the relationship between antigens and antibodies. It soon became one of the cornerstones of 20th-century immunology.83 Ehrlich’s other major contribu- tion was to point out the difference between active and passive immunity.13,84

The last decade of the 19th century produced remarkable advancements from remarkable men. von Behring was awarded the first Nobel Prize in Medicine (1901); Koch received it in 1905, and Ehrlich and Metchnikoff shared the Nobel in 1908.

# FIRST HALF OF THE 20TH CENTURY

At the beginning of the 20th century, five human vaccines were in use: Jenner’s original smallpox vaccine and Pasteur’s rabies vaccine (both containing live virus) and three bacterial vac- cines: typhoid, cholera, and plague (all killed). In addition, immunization with diphtheria or tetanus antitoxin was an accepted practice. The 19th century’s end also saw the end of arm-to-arm lymph inoculation as a vehicle for smallpox vac- cination. This technique was replaced by the use of glycerin- ated calf lymph in 1898.13 Most of the fundamental concepts of vaccinology had been introduced by the end of the 19th century; the early 20th century would bring refinements to these theoretical underpinnings. Not until the advent of cell culture 50 years later would the field again become so dramati- cally fertile ([Table 1.1](#_bookmark5)).

Wright proposed mass immunization of British troops with killed typhoid vaccine during the Boer War (1899), but there was opposition because of adverse reactions and he was able to vaccinate only 14,000 volunteers. Opposition ran so high that consignments of vaccine were dumped overboard from transport ships in Southampton. The result was catastrophic: more than 58,000 cases of typhoid and 9000 deaths in the British Army.63 A bitter battle about the merits of the vaccine was waged in the *British Medical Journal* between Wright and the statistician Karl Pearson. Ultimately, at Wright’s insistence, the War Board initiated a broad-based trial that showed the overwhelming effectiveness of the vaccine. Wright was then knighted. By the beginning of World War I in 1914, general typhoid vaccination was conducted in the British Army, although it was still not mandatory.63,85,86

During the first few decades of the 20th century the use of

“bacterins” as human vaccines came into use. Bacterins con- sisted of killed bacteria (antigens) that were injected parenter- ally to produce active immunization. Sometimes they were combined with immune serum to become “serobacterins”— the serum would provide short-term immunity before the killed bacterial antigens kicked in to provide long-term immu- nity. The concept was an outgrowth of Metchnikoff’s theory of phagocytosis and Wright’s work on opsonins. It was thought that bacterins worked because opsonins induced by the anti- gens prepared invading bacteria for phagocytosis. Most bac- terins were prepared and sold without clinical trials. In its 1908 catalog, HK Mulford Company, a forerunner to Merck, listed nine bacterins that it sold, including those for gonor- rhea, typhoid, pneumococcal disease, and streptococcal disease. Bacterins for humans fell into disuse by the late 1930s as more stringent licensing requirements were imposed by the federal government.87,88 Bacterins for specific herds are still used as targeted vaccines for animals.

In the early 20th century, the *chemical* inactivation of diph-

theria and other bacterial toxins led to the development of the first **toxoids**: diphtheria and tetanus. Here again, Theobald Smith had a significant role. In 1907, he determined that toxoids provided immunity in guinea pigs. In a 1909 report

on long-lasting immunity against diphtheria in guinea pigs immunized with toxoid, he suggested that toxoids should be considered for humans.59,89

In 1923, Alexander Glenny and Barbara Hopkins showed that diphtheria toxin could be transformed into a toxoid by formalin.90 The discovery came about when the containers in which the batches of diphtheria toxin were kept were cleaned with formalin (they were too large to be autoclaved). The residual formalin in the vats rendered the batch of toxin so weak that 1000 times the normal dose did not kill the guinea pigs. Although this toxoid was certainly safer than the toxin, it could be administered only in conjunction with antitoxin. In that same year, Gaston Ramon developed a diphtheria toxoid that could be used on its own (i.e., without antitoxin) by adding formalin and incubating the mixture at 37°C for several weeks.91 Ramon and Christian Zoeller used a tetanus toxoid developed in the same manner for the first human vaccinations against tetanus in 1926.92,93

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The vaccine against **tuberculosis,** bacille Calmette-Guérin

(BCG), was the first live vaccine for humans to be produced since Pasteur’s rabies vaccine in 1885. Calmette was a protégé of Emile Roux and founder of the Pasteur Institutes at Lille and in Indochina.13 In 1906, Calmette and Camille Guérin, a veterinarian, started subculturing a strain of mycobacteria obtained from a bovine, which they perhaps thought was *Mycobacterium tuberculosis* but was in reality *Mycobacterium bovis*. They originally focused on producing a serotherapy, along the lines of von Behring’s antidiphtheria serotherapy, but quickly realized they could not inhibit the pathogenicity of the bacillus very easily. That began their pursuit of a vaccine. After 13 years of attenuation by 230 passages in beef bile, potatoes, and glycerol, this strain eventually became the BCG strain. In total, Calmette and Guérin spent more than 20 years trying to understand the mechanism of infection of tubercu- losis.94 Clinical trials in children began in 1921, and the vaccine became available for human use in 1927.13,95–99 Because the original vaccine strain was sent to numerous laboratories around the world, each of which then produced its own varia- tion of BCG vaccine, standardization has proved difficult. Despite the existence of more than a dozen major BCG vaccine strains that vary widely in strength, BCG remains an effective, if imperfect, vaccine against tuberculosis in children.100

In 1931, E.W. Goodpasture introduced the use of the cho-

rioallantoic membrane of the fertile hen’s egg as a medium for growing viruses.55,101 This technique represented a major advance because until then it was thought that human viruses could be grown only in animals such as ferrets and mice. Ferrets were expensive, and mouse brain could produce aller- gic brain encephalitis. The chick embryo proved to be a cheaper and safer medium for the cultivation of viruses. Earlier, in the second Milroy Lecture of 1898, Copeman described conducting an experiment using hens’ eggs— successfully—to grow vaccinia virus for the production of smallpox vaccine.34,102

**Yellow fever** virus was isolated in 1927 by two independent groups: researchers at the Rockefeller Foundation working in Nigeria, who isolated the Asibi strain,103–105 and researchers at the Pasteur Institute in Senegal, who isolated the French strain.106,107 The French strain was given to various research

groups for study.107 In 1928, A.W. Sellards at the Harvard Medical School began collaborative research on the French strain with Jean Laigret at the Pasteur Institute in Senegal. Max Theiler, working for Sellards at Harvard, developed an animal model to study the virus.108 Using passage in mouse brain, others were able to “fix” the neurovirulence of the strain,109 which then was used as a vaccine. This French strain yellow fever vaccine from Theiler’s work at Harvard was a live vaccine derived from mouse brain passage.110 Sellards and Laigret

|  |  |  |  |
| --- | --- | --- | --- |
| **TABLE 1.1** Outline of the Development of Human Vaccines (Wherever Possible, Date of Licensure Is Indicated) | | | |
| **Live Attenuated** | **Killed Whole Organism** | **Native Protein or Polysaccharide** | **Genetically Engineered** |
| **18th Century** |  |  |  |
| Smallpox (1798) |  |  |  |
| **19th Century** |  |  |  |
| Rabies (1885) | Typhoid (1896) |  |  |
|  | Cholera (1896) |  |  |
|  | Plague (1897) |  |  |
| **20th Century, First Half** |  |  |  |
| Tuberculosis (bacille Calmette- Guérin) (1927) | Pertussis (1926) | Diphtheria toxoid (1923) |  |
| Yellow fever (1935) | Influenza (1936) | Tetanus toxoid (1926) |  |
|  | Typhus (1938)  Tickborne encephalitis (1937) |  |  |
| **20th Century, Second Half** |  |  |  |
| Polio (oral) (1963) | Polio (injected) (1955) | Pneumococcus polysaccharide (1977) | Hepatitis B surface antigen recombinant (1986) |
| Measles (1963) | Rabies (cell culture) (1980) | Meningococcus polysaccharide (1974) | Lyme OspA (1998)b |
| Mumps (1967) | Japanese encephalitis (mouse brain) (1992)b | *Haemophilus influenzae* type b polysaccharide (1985)b | Cholera (recombinant toxin B) (1993) |
| Rubella (1969) | Tickborne encephalitis (1981) | Meningococcal conjugate (group C) (1999) U.K.a |  |
| Adenovirus (1980) | Hepatitis A (1996) | *H. influenzae* type b conjugate (1987)a |  |
| Typhoid (*Salmonella* Ty21a) (1989) | Cholera (WC-rBS) (1991) | Hepatitis B (plasma derived) (1981) |  |
| Varicella (1995) |  | Typhoid (Vi) polysaccharide (1994) |  |
| Rotavirus reassortants (1999) |  | Acellular pertussis (1996) |  |
| Cholera (attenuated)b (1994) |  | Anthrax secreted proteins (1970) |  |
| **21st Century** |  |  |  |
| Cold-adapted influenza (2003) | Japanese encephalitis (2009) (Vero cell) | Pneumococcal conjugates (heptavalent) (2000)a | Human papillomavirus recombinant (quadrivalent) (2006) |
| Rotavirus (attenuated and new reassortants) (2006)  Rotavirus (monovalent) (2008)  Cholera (oral) (2016) | Cholera (WC only) (2009) | Pneumococcal conjugates (13-valent) (2010) | Human papillomavirus recombinant (bivalent) (2009)  Human papillomavirus (9-valent) (2014)  Meningococcal type B (fH factor) (2014)  Meningococcal type B (reverse vaccinology) (2015) |
| Zoster (2006) |  | Meningococcal conjugates (quadrivalent) (2005)a |  |
| aCapsular polysaccharide conjugated to carrier proteins.  bNo longer available. | | | |

intended to do the human trials on the yellow fever vaccine at the Institut Pasteur in Paris, but Roux, who was then the director, refused to allow the human trials with the murine virus to be conducted. He thought it was too dangerous.111 Eventually it was used in humans without immune serum by Sellards and Laigret in 1932.112 However, owing to the strain’s passage through mouse brain tissue, the neurovirulence of the French strain did indeed present grave dangers.

Theiler subsequently left Harvard to join the Rockefeller Institute and attempted to develop a more attenuated vaccine, using the Asibi strain. Theiler and Hugo Smith developed the 17D strain from Asibi in fertile hen’s eggs chorioallantois per

Goodpasture’s method. Although the French strain was also highly effective, the 17D strain was both effective and much safer.55,107,113–115 The French strain certainly saved many lives, especially in French West Africa, where it was used extensively. It remained in production (in modified form) until 1982; however, safety concerns about the use of mouse brain tissue overrode its proven efficacy and 17D won out as the vaccine strain of choice.107 For this work, Theiler was awarded the Nobel Prize in 1951.

Wilson Smith, Christopher Andrewes, and Patrick Laidlaw isolated human **influenza A** virus in ferrets in 1933.116 They followed the technique outlined by Richard Shope of the

Rockefeller Institute when he isolated the swine influenza virus in pigs in 1931. Within 5 years, Smith’s group and Shope were able to show that swine influenza virus was a surviving virus from the great influenza pandemic of 1918.117,118 Frank Horsfall, Alice Chenoweth, and colleagues developed a live influenza virus vaccine in mouse lung tissue in 1936.119,120 Chenoweth claimed that it became inactivated or nonreplicat- ing when it was administered parenterally.120,121 That same year, 1936, saw the development of two influenza A vaccines grown in embryonated eggs, one (live) by Wilson Smith122 and the other (killed, whole virus) by Thomas Francis and Thomas Magill.123,124 Even though these two vaccines were considered safer because they were developed in embryonated eggs, Che- noweth’s mouse lung vaccine had contained a higher virus yield and was the first to demonstrate true protection in humans, albeit transient.

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In 1937, Anatol Smorodintsev and colleagues in the Soviet

Union administered the Wilson Smith strain to humans by the intranasal route, using doses that were lethal when given to mice.125 This is considered the first *live* human influenza virus vaccine, and although it would not receive a passing grade by today’s standards (20% of vaccinees developed febrile influenza), it absolutely demonstrated the role of the virus in the development of influenza.121,126

Frank Burnet and D.R. Bull showed in the early 1940s that live attenuated influenza virus could be produced in embryo- nated eggs but also that the resultant virus mutated rapidly. Therefore, the vaccines that were produced were not consis- tently attenuated and often produced disease.127,128 By con- trast, the Francis and Magill killed, whole-cell influenza A vaccine did not have the problem of mutated viruses and did not produce disease.

In 1940, Francis and Magill independently isolated **influ- enza B;** at this point, it was recognized that at least three strains of influenza were circulating at the same time. Francis devel- oped a killed (formaldehyde), trivalent (2As, 1B) vaccine that was mass-produced for the U.S. military in World War II. This conferred a certain “legitimacy” to killed influenza vaccine, as the military did not have to be concerned about “down time” from live vaccine–associated influenza. During this same period, Burnet developed a live aerosolized influenza vaccine, but the influenza season had already begun, so an efficacy trial was inconclusive. By then, the killed vaccine of Francis had been very successful during the war. The Australian govern- ment denied Burnet permission to continue trials of a live vaccine as too risky.121,126,129,130 Except for the Soviet Union, which continued to use live vaccine, killed influenza vaccine became the standard until the 1990s (see subsequent text).

During the 1947 flu season, the influenza vaccine did not protect, definitively proving the concept of antigenic variation of the virus strain from year to year, which had first been proposed by Magill and Francis in 1936. The term itself, “anti- genic drift,” was introduced by Burnet in 1955 in his *Principles of Animal Virology*.126

Many attempts were made to develop vaccines against rick- ettsiae once Charles Nicolle had discovered in 1909 that they were the cause of **typhus**.13 The first truly successful typhus vaccine was developed in 1938 by Herald Cox,131 who used the yolk sac of the chick embryo to grow *Rickettsia rickettsii*. Cox was working on Rocky Mountain spotted fever at the time, but once he found a method to cultivate the rickettsia, killed vaccines for typhus and Q fever quickly followed. There was a heavy demand for the typhus vaccine during World War II.13,132,133

Jules Bordet and Octave Gengou first observed the causal agent of **pertussis** in 1900 and cultivated it by 1906.13,134 Several vaccines were tested in small trials. Thorvald Madsen later carried out the first controlled clinical trials of a pertussis

vaccine (i.e., whole killed organisms) on the Faeroe Islands in 1923–1924 and again in 1929.135,136 During the 1923–1924 epidemic, Madsen reported that the vaccine did not prevent disease but greatly reduced mortality and severity of illness among vaccinated persons. By the 1929 epidemic, the vaccine had been considerably improved but still did not prevent disease.137 In the 1930s, Pearl Kendrick and Grace Eldering ([Figs. 1.6](#_bookmark8) and [1.7](#_bookmark9)), working for the Michigan Department of



**Figure 1.6.** Pearl Kendrick. *(From Grand Rapids History & Special Collections, Archives, Grand Rapids Public Library, Grand Rapids, MI.)*

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**Figure 1.7.** Grace Eldering. *(From Grand Rapids History & Special Collections, Archives, Grand Rapids Public Library, Grand Rapids, MI.)*

Health, improved the yield of the Bordet-Gengou growth medium and developed a killed (thimerosal) vaccine that they successfully tested in more than 1500 children. Only 4 of 712 vaccinees developed mild cases of whooping cough. They recruited the help of Eleanor Roosevelt to gain additional funds for further research and by 1940, their vaccine was distributed throughout the United States.138 The American Academy of Pediatrics approved the vaccine in 1943 and the American Medical Association in 1944. Several whole-cell per- tussis vaccines were in use by the late 1940s.139,140 The first **combination vaccine,** DTP (diphtheria, tetanus, pertussis) became available in 1948.141

# SECOND HALF OF THE 20TH CENTURY TO THE PRESENT

The latter half of the 20th century can truly be called the Golden Age of vaccine development. This occurred primarily because of the ability to grow viruses in stationary **cell culture**. Hugh and Mary Maitland from Manchester University first developed the flask **tissue culture** technique in 1928.13,59 They succeeded in growing vaccinia virus in sterile cultures of minced rabbit kidney in rabbit serum and mineral salts—a great accomplishment that proved to be a turning point in virus research. George Gey improved the virus yield of this method by continually rolling the tubes, thus increasing the oxygenation of the cells.59

After the Second World War, John Enders, Thomas Weller, and Fred Robbins took up research on cell culture at Boston Children’s Hospital. After using tissue cultures of the Maitland type (substituting monkey kidney for Maitland’s rabbit kidney), they tried to grow viruses in explanted human cells, using fibroblasts grown from the foreskin and muscle tissue of infants who had died soon after birth. Their first success was to grow Lansing type II poliovirus in human cell culture.142 The ability to grow human viruses in vitro, in a relatively easy and safe manner in monolayer cell cultures, led to an veritable explosion of creativity in vaccinology that continues unabated (see [Table 1.1](#_bookmark5)). Indeed, Enders, Weller, and Robbins received the Nobel Prize in Medicine in 1954 for their seminal work. Paralytic **polio** became an epidemic problem only in the latter half of the 19th century when epidemics broke out in Western Europe and the United States.143 After the virus was isolated by Landsteiner and Popper,144,145 there were attempts to make polio vaccines in the 1930s, especially by John Kolmer and Maurice Brodie.146–148 These vaccines were poorly and hastily tested, and their use resulted in at least six deaths and numerous cases of vaccine-associated paralytic polio. The backlash against these ill-conceived experiments was swift. At the meeting of the American Public Health Association in St. Louis in November 1935, James Leake, medical director of the

U.S. Public Health Service, directly accused Kolmer of murder!

Kolmer stopped working on vaccines after this disastrous inci- dent and returned to Temple University where he taught Public Health and Preventive Medicine.31,146 Polio vaccine development for the most part ground to a halt for the next 15 years.146

A rare exception to this hiatus was Hilary Koprowski, who did work on polio vaccine during this period and developed the first live oral polio vaccine (OPV) with an attenuated polio variant virus strain grown in mice, not cell culture. It was tested in humans in 1950.149

The logjam preventing further polio vaccine research was broken with the aforementioned report by Enders, Weller, and Robbins in *Science* in 1949.142 All subsequent research on polio vaccine was based on attenuation or passage in cell culture. In rapid succession thereafter, articles appeared

showing that the Lansing strain of polio could be grown in a variety of human and other primate tissues,150–155 and several laboratories began research on polio vaccines.146 Thus began **“The Polio Wars.”**

The first licensed product developed using the cell culture technique was the trivalent, formalin-inactivated polio vaccine (IPV) of Jonas Salk, licensed in 1955.156 Salk gained his vaccine experience under his mentor Thomas Francis, with whom he developed the first killed influenza vaccine in 1938, which was used to vaccinate the American troops during the Second World War. The majority of Salk’s research on killed polio vaccine and the large clinical trial in 1954 (more than 1.8 million children) were funded by the National Foundation for Infantile Paralysis (March of Dimes), founded by Franklin Roosevelt in 1938 and directed by Basil O’Connor. The Foun- dation’s idea on the trial design did not include a placebo- control; on the contrary, Francis insisted on the need for placebo-control. After a shaky start, the clinical trial was designed by a committee that included both points of view, headed by Thomas Francis, Salk’s former mentor, who ulti- mately directed the trial. Although Salk wanted every child in the trial to receive his vaccine, Francis stood his ground and insisted on use of a placebo. The controversial trial design was called a “dual-control” trial. In 11 states, children in grades 1 through 3 received injections of either the polio vaccine or a placebo. In 33 other states, children in grade 2 received injec- tions of the vaccine and all children from grades 1 through 3 were “observed” for the duration of the polio season. In the end, 420,000 children received Salk’s vaccine, 200,000 a placebo, and 1.2 million were “observed.”146,157,158

The quest for a polio vaccine was highly publicized, adding

pressure on everyone involved to hurry up and develop a vaccine. Although Salk’s public persona generated a lot of animosity from professional colleagues who saw him as a publicity hound, when the overwhelmingly positive results of Salk’s trial were announced on April 12, 1955, with great fanfare, ordinary Americans were ecstatic and Salk became an instant “hero.” The inactivated Salk vaccine was immediately licensed, and within days, six companies had been granted rights to manufacture the vaccine.143,146,159

This haste was understandable, as the “summer polio season” was about to begin and parents were anxious to vac- cinate their children. But it was short sighted. Shortly thereaf- ter, the Cutter Incident occurred,146 in which cases of paralytic polio were detected in vaccine recipients. Epidemiologic evi- dence showed that the product of one manufacturer, Cutter Laboratories, was implicated in most of these vaccine-related cases. The nation’s entire polio vaccination program was put on hold until a review of manufacturing procedures could determine the source of the problem.146,160,161 More rigorous safety testing was put in place and an additional filtration process added to remove clumps of partly inactivated virus before the vaccination program resumed. The contaminated vaccine resulted in 260 cases of paralytic polio and 10 deaths in recipients, their families, or community contacts. Only one manufacturer was implicated, although several had experi- enced problems. Because polio was such a widespread and feared disease, there was no lasting boycott of the vaccination program.143,146

Despite the success of Salk’s killed vaccine, others contin-

ued research to develop *live* attenuated polio vaccines in cell culture: Herald Cox at Lederle, Koprowski at the Wistar Insti- tute, and Albert Sabin at Cincinnati Children’s Hospital. Like Jenner and Pasteur, these researchers believed that an infection with live virus would provide more long-lasting immunity and greater resistance to disease.143 Because Salk’s vaccine was already in use, Sabin was unable to get funding in the United States for the required clinical trial to prove the efficacy of a

live polio virus vaccine. Despite the Cold War, and probably because he was born in Russia, Sabin was able to collaborate with Russian scientists to do the enormous clinical trials in the Soviet Union. Based on Dorothy Horstmann’s favorable review of the Russian trial results, Sabin’s vaccine was licensed in the United States in 1960. Until 1963, both vaccines (Salk’s killed and Sabin’s live) were used in the United States. In 1964, the Committee on the Control of Infectious Diseases of the American Academy of Pediatrics voted a clear preference for Sabin’s live OPV, which was grown in monkey kidney cell culture and was easier to administer (vaccine on a sugar cube). As a result, it came into wide use.143,162

For the next 30 years OPV was the primary recommended vaccine in the United States and in many, but not all, Euro- pean countries (Sweden, Finland, and the Netherlands continued using IPV). Although there were reports of vaccine- associated cases of polio with OPV, Sabin adamantly defended his vaccine and refused to believe that it was capable of causing polio. But by the 1990s, the only cases of polio occurring in the United States and Europe were vaccine-associated, caused by mutation of the live virus and reversion to virulence in OPV. In the year 2000, the United States switched to the exclu- sive use of IPV.

Thanks to both of these vaccines, polio has been eradicated from the Western Hemisphere, and the World Health Organi- zation (WHO) has targeted polio to be the next disease after smallpox to be effaced from the entire world. In the May 2015 WHO report, the recommendation was made to remove Type 2 polio strain (the strain that causes most vaccine-associated polio) from all OPV vaccine by April 2016 and to begin intro- duction of IPV wherever possible by that same date.163

Many others took advantage of cell culture techniques. Samuel Katz, Milo Milanovic, Enders, and colleagues devel- oped the Edmonston strain of **measles** vaccine, grown in chick embryo cell culture,164 which was attenuated further by Maurice Hilleman and colleagues165 and by Anton Schwarz.166 Hilleman also attenuated the Jeryl Lynn strain of **mumps** virus (obtained from and named after his own daughter Jeryl Lynn) in the hen’s egg and obtained licensure in 1967.167 Rubella virus was isolated in 1962, both by Tom Weller168 and Parkman and associates.169 The virus was attenuated by passage in cell culture and by 1970, several strains had been developed and were in use: in monkey kidney by Paul Parkman and Harry Meyer (further passaged in duck embryo cells by Hilleman),170 in rabbit kidney by Abel Prinzie and Constant Huygelen,171 and in human fibroblasts by Stanley Plotkin.172 The latter strain (Wistar-RA27/3), the first vaccine made in human fibro- blasts, is the sole rubella vaccine in wide use since 1980 because of its better safety and efficacy. In 2015, the Pan American Health Organization (PAHO) announced that rubella was officially declared eliminated from the Western Hemisphere.2,173

In the 1950s, the **adenoviruses** were first recovered from adenoids that had been surgically removed.174 Until their iso- lation, a great variety of the illnesses they cause were attributed to other diseases. Formalin-inactivated, whole-virus vaccines were made by Hilleman against types 4 and 7; they were licensed for military use only. In the early 1960s, the seed stock was shown to be contaminated with simian virus 40 (SV40)175; when all attempts to eliminate SV40 failed, the vaccine was withdrawn in 1963.176

Vaccine studies continued, however, using human embryo kidney (HEK) cells and subsequently human diploid cell strains, thus eliminating the SV40 problem. An enteric-coated vaccine tablet was produced for types 4, 7, and 21 by Chanock and colleagues177–181; the vaccine was licensed, but again, only for military use. Production ceased in 1996, primarily because of regulatory issues and lack of interest by the military. Not

unexpectedly, within a few years significant outbreaks of ade- novirus respiratory diseases reoccurred in military recruits. After several years, the U.S. Army signed a contract with another producer to reformulate the original vaccine.182 In 2011, the U.S. Food and Drug Administration (FDA) again approved a live oral vaccine for adenovirus types 4 and 7 for military populations ages 17 to 50 years.

The live attenuated Oka strain of **varicella** vaccine was developed in the 1970s by Michiaki Takahashi183,184 and underwent extensive clinical trials before being licensed in Japan and several European countries.185,186 After a long and convoluted development, licensure was obtained in the United States in 1995.187 It is now recommended for all children older than 1 year.188 As of 2009, there has been a 90% decline in varicella-related hospitalizations.189–193

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The discovery of the relationship between chickenpox and shingles goes back to the late 19th century. A Hungarian sci- entist, James Bokey, described cases of chickenpox in persons who had been in contact with someone with shingles.31 Some 30 years later, in an experiment that certainly could not be done today, a German scientist named Kundratitz proved him right by inoculating children with shingles “pus!” They devel- oped chickenpox.31,194,195 Thus, the attenuated Oka strain in high concentrations could be used as a zoster vaccine.

In May 2006, the FDA granted a license for Zostavax, a higher-potency, live attenuated Oka virus vaccine against **zoster**.196 This higher-dose vaccine reduces the incidence of shingles and also reduces the severity of postherpetic neuralgia in those cases of shingles that occur in vaccinated persons.197–201 Originally recommended for those age 60 years and older, it is now recommended for anyone older than age 50 years. A new zoster vaccine based on a viral glycoprotein may be licensed soon.

Influenza vaccines were first licensed in the United States in 1945202; the first recommendation for their inclusion in the pediatric schedule did not occur until 2004. By the 1960s, a **live influenza vaccine** that was safe for adults was achieved, the Alice strain (H3N2),203,204 but it could not be used in chil- dren, who became febrile.205 The annually administered *killed* influenza vaccine, which had consistently proved to be safe and effective but without lasting local or cellular immunity, remained the standard.

Newer vaccine design technologies in the 1990s, including reassortment, reverse genetics, and cold adaptation, have again made it possible to develop *live* attenuated influenza vaccines that confer long-term immunity and obviate the need for injections.

Three attenuated master strains were developed for live influenza vaccines: host-range, temperature-sensitive, and cold-adapted mutants. Only the cold-adapted influenza vaccine developed by Hunein Maassab has been licensed in the United States (2003).206 Other strains were attempted and abandoned because of inconsistent attenuation, instability, and, occasionally, reversion to virulence.207 Cold-adapted strains allow the vaccine virus to grow in the relative coolness of a subject’s nasal passages (32°C) but not in warmer internal organs, particularly the lungs (37°C).208

Live attenuated influenza vaccine (LAIV) is administered by nasal spray and is a good example of the advantages of **reas- sortant technology**, which is possible with viruses that have segmented genomes. Coinfection of cell culture with wild and attenuated strains allows the mixing of genome segments and identification of viruses containing genetic material from both strains. Each year, new influenza vaccine strains for LAIV are made by reassorting the six internal genes from Maassab’s master strains with the genes coding for the hemagglutinin and neuraminidase surface glycoproteins of circulating wild strains of influenza viruses.207,209 (New vaccine strains for *killed*

influenza vaccine also are produced each year by reassortment or by recombinant technology.) The live vaccine was originally shown to be as effective as the killed vaccine in children and offered longer and broader immunity.207,210–214 Furthermore, the live vaccine is easy to administer and has shown cross- protection against antigenically drifted wild strains.215,216 However, recent LAIV strains have shown poor immunogenic- ity, and the vaccine’s utility is currently uncertain.

An orally administered, quadrivalent, live **rotavirus** reas- sortant vaccine developed by Albert Kapikian and associates, was licensed for use in the United States in September 1998.217–219 Within 10 months of licensure, cases of intussusception among vaccine recipients were reported to the Vaccine Adverse Event Reporting System.220–222 Based on epidemiological studies, the Centers for Disease Control (CDC) determined that intussusception occurred with significantly increased fre- quency within the first 2 weeks after vaccination.223 The vaccine was formally withdrawn from the market in November 1999.224

Other rotavirus vaccines were developed by H. Fred Clark, Paul Offit, and Stanley Plotkin at the Wistar Institute and the Children’s Hospital of Philadelphia and by Richard Ward and David Bernstein at Cincinnati Children’s Hospital. The Wistar/ Children’s Hospital of Philadelphia rotavirus vaccine is based on a bovine rotavirus (WC-3), attenuated for humans, which has been reassorted with five human rotavirus RNA segments that code for vp4 or vp7 protein, from different serotypes,225,226 thus producing a pentavalent oral vaccine, which was licensed for use in the United States in 2006.227 The Cincinnati group developed a vaccine based on a single human rotavirus attenu- ated by passage in cell culture that contains serotypes G1 and P1a[8]. It was licensed in the United States in 2008.228–230

Because concern about intussusception remained strong, both of these vaccines underwent enormous clinical trials (more than 60,000 were tested in each case) to determine whether an association existed. Both vaccines proved highly efficacious, and intussusception was rare.231–234 Clinical trials expanding the use of these vaccines to developing countries show protection, although not as high as in developed coun- tries. This has led to a global recommendation for the use of rotavirus vaccine by the WHO.235–239

Parenteral killed cholera vaccines did not gain wide accep- tance because of limited efficacy, fleeting protection, and side effects and were eventually pulled from the market. Several oral vaccines against cholera and its debilitating diarrhea have been developed: two killed and one live. The **killed cholera vaccine** developed by Jan Holmgren and colleagues, combines the whole cell of *V. cholerae* O1 and subunit B of the cholera toxin (WC-rBS).240,241 The second killed vaccine contains O1 and O139 strains but no recombinant subunit (WC-only). This makes it easier to administer and less expensive to produce. It is currently used primarily in India and Vietnam. Both killed vaccines are prequalified by the WHO, licensed in many countries, and used to prevent travelers’ diarrhea as well as for epidemic control.240–243

An attenuated **live oral cholera vaccine**, derived from Inada strain 569B has been developed by Myron Levine and colleagues at the University of Maryland.244–251 This vaccine requires a cold chain and a buffer. The vaccine was licensed and sold in many countries; it offered rapid onset of protec- tion. For reasons unrelated to the vaccine itself, the manufac- ture was interrupted (bankruptcy proceedings and company takeovers). Ultimately, the rights to the vaccine were returned to the University of Maryland. In 2015, a biotech company reached an agreement with the University of Maryland to bring the live oral cholera vaccine to FDA licensure. In 2016, the vaccine was approved for use in adults aged 18 to 64 years traveling to cholera-affected areas.251a

After the early work on killed **typhoid vaccine**, a variety of heat-phenol–killed or acetone-killed, parenteral, whole-cell typhoid vaccines became available.252–255 All had high rates of adverse reactions and were never considered quite satisfactory. An important advance was made by René Germanier and E. Fürer when they developed an attenuated strain of the Gal E mutant Ty21a of *Salmonella typhi*.256 After preliminary vaccine studies in the United States,257 large trials were conducted suc- cessfully in Egypt258 and Chile.259,260 Protection rates varied; however, there were few adverse reactions, and oral formula- tion of this vaccine made it less expensive to produce and distribute.261,262 Typhoid Vi *polysaccharide*, a killed, purified- component vaccine was developed by Landy, Webster, and colleagues263–265 and later improved by Wong and associates266 and John Robbins and J.B. Robbins.267

The adaptation of **rabies** virus to human diploid cell culture

permitted the development of a potent, whole-virus inacti- vated rabies vaccine by Koprowski, Tadeusz Wiktor, and asso- ciates.268 This vaccine is much more immunogenic than prior rabies vaccines. Since then, many other cell culture rabies vac- cines have been developed, including a vaccinia-recombinant rabies vaccine for veterinary use.269

The development of a **Japanese encephalitis** (JE) vaccine was attempted during the Second World War,270 but later a formalin-inactivated whole-virus vaccine, harvested from mouse brain, was developed in Japan in 1965.271 It was put into use almost immediately to vaccinate Japanese chil- dren, although few data regarding its efficacy had been published.

After two Americans who had traveled in Asia died of JE, the U.S. Department of Defense conducted a vaccine trial in northern Thailand272 that showed an efficacy of 91%. A biva- lent vaccine, also in mouse brain, was developed using the Nakayama-NIH strain (from the original vaccine) and the Beijing-1 strain to provide immunity to strains from different geographic areas.272–274

X.Y. Yu and coworkers developed live attenuated and inac- tivated vaccines against JE, each in primary hamster kidney cells.275–279 The Chinese live vaccine is widely used in Asia, but hamster kidney cells are not approved by the WHO.280

The bivalent, inactivated, mouse-brain–derived vaccine needed improvement. An increase in allergic reactions was noted in the 1990s, several doses were required to maintain immunity, and it was developed in animal nervous tissue (mouse brain).280 In March 2009, the FDA approved a second- generation JE vaccine for adults that is Vero-cell produced, inactivated, and purified. Ixiaro uses the SA14-14-2 strain of virus that had been used in the Chinese live vaccine.281 In May 2013, the license was extended to include children older than 2 months to 16 years of age. It is the only licensed JE vaccine in the United States; the previous one, made in mouse brain tissue, ceased production in 2006.281–284

In 1979, Maurice Hilleman and Phil Provost grew **hepatitis A** virus (HAV) in cell culture,285 opening the path for the development of a vaccine. Hilleman, Provost, and coworkers developed the first inactivated HAV vaccine in 1986286; however, the cell culture used to produce the HAV antigen was not suitable for use in humans. Formaldehyde-inactivated, whole-virion HAV vaccines grown in human fibroblasts were later developed and licensed in the United States in 1995– 1996.287,288 By 2004, HAV infection rates declined to 1.9 per 100,000 population, the lowest rate ever.289 Universal HAV vaccination for all children was recommended in 2006.290

The first killed **tickborne encephalitis** (TBE) vaccine, pro- duced in mouse brain, was developed in the Soviet Union in 1937, shortly after the virus was identified and the tick vector verified.291,292 In the 1960s, basing their work on Benda and Danes,293,294 two separate groups, Levkovich in the Soviet

Union295 and Kunz in Austria,296 used chick embryo cell culture to develop less-reactogenic, formalin-inactivated vac- cines. A **whole virus inactivated vaccine** was developed by Heinz, Kunz, and Fauma in 1980,297 and is effective against isolates of the TBE virus that share a homologous envelope glycoprotein.298–300 Since 1999, this vaccine has undergone several changes in the manufacturing process that have improved its purity, especially a switch from mouse brain protein to chick embryo cells.301 A second inactivated TBE vaccine was licensed in Germany in 1991.302 After the collapse of the Soviet Union in 1989 and the opening of Eastern Europe, the geographic range of TBE was shown to be quite large; it is now endemic to most European countries, the Russian Federation, and several Asian countries, including China, Japan, and South Korea.

It is clear that by the late 1970s, another transition was occurring in the development of vaccines. Technology had advanced to the point that scientists could identify many com- ponents of the infectious agent, viral or bacterial. A new cat- egory of vaccines emerged, based on bacterial proteins, polysaccharides, and protein-conjugated polysaccharides. Live attenuated and killed whole-cell vaccines were still important but they did not apply to all diseases. Subunit vaccines were created where the protective antigen could be identified and isolated. Some whole-cell vaccines were replaced by subunit vaccines.

Whole-cell pertussis vaccine from the 1940s caused a number of adverse reactions, mostly mild, but some more serious. In 1975, when two deaths occurred in children shortly after they received whole-cell pertussis vaccine, the Japanese Ministry of Health suspended its use, although a causal rela- tionship was not established. An astronomical increase in the incidence of pertussis followed: 206 reported cases in 1971 grew to 13,105 cases by 1979. Realizing that it had overre- acted, the Ministry of Health reinstated use of the vaccine, but only for children older than 2 years.303 Similar problems occurred in the United Kingdom, where vaccination rates fell to less than 33% by 1977. Three major epidemics followed, causing more than 100,000 cases of whooping cough and 36 deaths.304 The adverse reactions associated with whole-cell per- tussis vaccine led to the development of a Japanese **acellular pertussis** vaccine by Yuji Sato and Hiroko Sato303,305,306 that is less reactogenic. Based on two of the main protective antigens of *Bordetella pertussis,* toxin and filamentous hemaggluti- nin,303,305,307 it was licensed for use in Japan in 1981. Since then, other acellular pertussis vaccines, containing one to five protective antigens, have been licensed in the United States (1996) and other countries.308 However, despite routine vac- cination, an increase in cases of pertussis since 2005 has led to the reevaluation of the acellular vaccines, which give less- persistent immunity. New formulations are being considered to make the acellular vaccine, as well as the use of more fre- quent boosters.

Modern work on a human **anthrax** vaccine began in the second half of the 20th century. The vaccine, named anthrax vaccine adsorbed (AVA), contains the secreted protein called “protective antigen” that forms part of the toxin. This was obtained from sterile filtrates of an attenuated, unencapsu- lated, nonproteolytic strain of *Bacillus anthracis*.309–311 A ran- domized field study on a similar anthrax vaccine took place from 1955 to 1959 at four mills that processed raw goat hair destined for the clothing manufacturing industry.312,313 AVA was licensed for use by the U.S. Army in 2002.

Human anthrax had not been viewed as a serious problem in the late 20th century. Worldwide, there were fewer than 2000 cases annually, mostly cutaneous, in the 1980s and 1990s.314 The bioterrorism incident in 2001, when highly refined anthrax spores were sent through the U.S. postal

system, changed that perception. It was considered prudent to ensure the availability and safety of the supply of anthrax vaccine for the military and the general public.315 Although the FDA has affirmed that the current vaccine is effective, no matter what the route of infection,316 research has been accel- erated to find a new-generation anthrax vaccine based on protective antigen made by recombinant technology or using newer adjuvants. Modern anthrax vaccines will require fewer injections to attain full immunity.317,318

During the 1970s and 1980s, several bacterial vaccines con- sisting of *purified capsular polysaccharides* were developed.

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**Meningococcal vaccines** had been made in the 1940s but failed to protect.319 Modern work on humoral immunity to meningococcal disease got underway at the Walter Reed Hospital in 1966, ultimately resulting in *capsular polysaccha- ride vaccines* against meningococcal serogroups A and C by Malcolm Artenstein,320 Emil Gotschlich,321 and associates.320–324 By the early 1970s, serogroup C meningococcal polysac- charide vaccine was routinely administered to U.S. Army recruits and had virtually eliminated the disease within the military.318 However, the vaccine did not provide immunity to children younger than 2 years, and the duration of immunity was uncertain. Additional boosters resulted in *reduced* immune

responses.325,326

Several serogroup A and/or serogroup C meningococcal *conjugates* were developed, conjugated to diphtheria or tetanus toxoid; they provide longer immunity than the polysaccharide vaccine and immunity to children younger than 2 years.325–330 The serogroup C conjugate was licensed in the United Kingdom in 1999 and placed in the universal immunization schedule in November of that year.327 After extensive postlicensure studies in the United Kingdom,326,328–330 a quadrivalent conju- gate vaccine against serogroups A, C, Y, and W135 was licensed in the United States in 2005 and a second one in 2010.331–334 It took less than 20 years from the time the **pneumococcus** was first isolated (in 1880, by Pasteur and George Sternberg simultaneously)335,336 to discover the multiplicity of pneumo- coccal serotypes and appreciate the complexity of developing a vaccine against it. A killed, whole-cell pneumococcal vaccine was made by Almroth Wright in 1911 and tested in South African goldmine workers, but eventually it was aban- doned.337,338 By the late 1940s, extensive work on capsular polysaccharides, notably by Colin MacLeod, resulted in a mul- tivalent capsular polysaccharide pneumococcal vaccine (four- valent and later six-valent).339–341 However, antibiotics were so

successful that the vaccine fell by the wayside.

The current **pneumococcal polysaccharide** vaccine was developed by Robert Austrian and associates.342 Austrian and Jerome Gold pointed out that pneumococcal disease contin- ued to be a severe problem despite the use of antibiotics.343 A modern capsular polysaccharide pneumococcal vaccine was subsequently developed for adults by Austrian, initially with 14 antigens (1977) and later increased to 23 antigens (1983).336,342

Although the pneumococcal polysaccharide vaccine was effective in adults, it did not protect children younger than 2 years, among whom more than 80% of invasive pneumococ- cal disease occurs; protein conjugation technology was applied to develop a vaccine that would protect this important group.344 A *heptavalent pneumococcal conjugate vaccine* (conju- gated to a nontoxic mutant of diphtheria) was produced and found to be safe, efficacious, and immunogenic in children younger than 2 years, who are the children most at risk.345,346 The vaccine was licensed in the United States in February 2000.347,348 A 1-year postlicensure follow-up study by Steven Black and associates demonstrated a dramatic reduction in age-specific invasive pneumococcal disease incidence.349 In addition, the use of the vaccine in children has exerted a

marked reduction in adult disease as the result of herd immu- nity. In February 2010, a 13-valent pneumococcal conjugate vaccine was licensed. PCV13 is recommended for prevention of pneumococcal disease in infants, elderly adults (>50 years of age), and immunocompromised individuals.350,351

The first-generation ***H. influenzae* type b** vaccine was devel- oped by Porter Anderson, David Smith,352 Rachel Schneer- son,353 and their associates. Richard Pfeiffer isolated this bacterium in 1892, but mistakenly thought he had found the causative agent of influenza. For years, it was known as Pfei- ffer’s bacillus before receiving its current name, *Haemophilus influenzae*.117 In the 1920s and 1930s, Margaret Pittman had determined that, of the six different polysaccharides of *H. influenzae*, organisms encapsulated with type b caused the largest proportion of serious disease in children. She identified the composition of the capsule as a polymer of ribosylribitol phosphate, now called polyribosylribitol phosphate (PRP).354 In the 1970s, several teams began research and efficacy studies on an *H. influenzae* type b vaccine, primarily in Finland and North Carolina.352,353,355–357 This work ultimately culminated in the 1985 licensure of the PRP vaccine.358 However, the vaccine was not effective for children younger than 18 months, who are most at risk for bacterial meningitis, and it had limited efficacy in older children. Vaccines against *H. influen- zae* type b bacteria advanced rapidly to second and third generations.

After it had been shown that the immunogenicity of a

capsular polysaccharide could be increased by binding it to a carrier protein,359,360 Schneerson and John Robbins linked *H. influenzae* PRP to diphtheria toxoid and developed the **first *conjugate* polysaccharide vaccine,** which was licensed in 1987.361 This vaccine had improved immunogenicity and effi- cacy and was licensed in 1987 for children older than 15 months. Younger children still remained at risk, but three more immunogenic conjugates soon followed, using nontoxic diphtheria toxoid (HbOC [haemophilus B oligosaccharide- CRM197]) derived from a mutant strain, an outer membrane protein of *Neisseria meningitidis* (PRP-OMP [polyribosylribitol phosphate–outer membrane protein]), or tetanus toxoid.358,361

# RECOMBINANT PROTEIN VACCINES

The discovery that the particles of **hepatitis B** surface antigen (HBsAg) found in infected people are immunogenic and pro- tective but noninfectious362–365 provided the basis for efforts to purify these particles from the blood of chronic carriers. Hille- man and colleagues succeeded in licensing a plasma-derived vaccine in the United States in 1981.365 Although the vaccine was safe and effective,366 the AIDS epidemic arrived at about the same time as the vaccine licensure; products derived from human blood were considered potentially dangerous. Despite rigorous safety testing and many inactivation processes to kill any foreign agent in the vaccine, the manufacturer could not overcome the reluctance of the public and physicians to use a product that had even a remote risk of containing the AIDS virus. Also, because the vaccine depended on human serum, sources of antigen were limited.

These obstacles prompted the formulation of the **first**

**recombinant vaccine,** HBsAg recombinant, which was licensed in 1986. This was accomplished by Valenzuela, Medina, Rutter, and others at the University of California, San Francisco, and the University of Washington in Seattle, by cloning the gene for HBsAg in yeast (*Saccharomyces cerevisiae*) and in mammalian cells. HBsAg was produced by the cells and then made into vaccine through adsorption on an alum adjuvant.367–370 In yeast, the surface antigen aggregated into particles similar to the extensively purified surface region antigen from the plasma-derived vaccine.371 Initial trials and

subsequent studies showed the recombinant vaccine to be as effective as the plasma-derived vaccine.369,372,373 In addition, because it is derived from a gene, it does not bear the stigma of possible contamination with undetected foreign agents.

**Lyme disease** was first recognized in the United States in 1975, and, within a quarter century, it became the most fre- quently diagnosed vector-borne disease in the country.374–377 Named after the town of Lyme, Connecticut, where it was first recognized, it was referred to as “Lyme arthritis” until 1982, when a constellation of associated illnesses was recognized and the name was changed to Lyme disease.378 Willy Burgdor- fer identified the spirochete causing the disease in the United States in 1982,379 and, subsequently, it was named *Borrelia burgdorferi*.

Personal protection, spraying, and antibiotics380–383 did not stem the rising tide of Lyme infection. Two vaccine candidates were put into extensive clinical trials.384–388 Each was based on a recombinant *Escherichia coli* strain containing the gene for outer surface protein A (OspA) of the American Lyme strain.375 An OspA vaccine was approved by the FDA in 1999 and rec- ommended for use in persons 15 to 70 years old who lived or worked in endemic areas of infection.377

Despite the extensive clinical trials and postlicensure sur- veillance, the vaccine was not well accepted because of a tepid recommendation from the CDC, the alternative of antibiotic treatment after infection, and the need for booster doses. Equally important, a series of class action and individual law- suits was brought against the manufacturer claiming that the vaccine caused chronic arthritis and other autoimmune prob- lems, although the evidence for vaccine-induced arthritis is absent.376 In April 2002, the vaccine was withdrawn from the market owing to lack of demand.389

A **recombinant, quadrivalent human papillomavirus** (HPV) vaccine was licensed in the United States in 2006, a landmark event because it represents the second vaccine against a human cancer (hepatitis B vaccine being the first) because it is 95% effective in preventing liver cancer caused by hepatitis B infection. The research to develop the HPV vaccine spanned at least 15 years, two continents, several laboratories, and competing claims of priority. The subunit vaccine is based on the capability of self-assembly of virus-like particles (VLPs) in the L1 (major) capsid protein, which is synthesized in yeast. Using HPV-16, an Australian group under Ian Frazer pub- lished data in 1991 showing the synthesis of L1 and L2 genes and the production of VLPs, but only when both L1 and L2 were synthesized together.390 They recognized that these VLPs could be a potential source material for a vaccine. One year later, John Schiller, Douglas Lowy, and others, at the National Cancer Institute, using a bovine model, established that the L1 capsid protein gene could self-assemble without the need for L2 and produced very high neutralizing antibodies.391 In effect, the L1 protein made itself into a structure that mim-

icked the HPV virion.

In 1993, Schiller’s group showed that HPV L1 produced in baculovirus was more efficient at self-assembly than the HPV L1 mutant that had been used by Frazer.392 Laboratories from Georgetown University and Rochester University also made significant contributions to the science underlying this vaccine.393,394 A concise summary of these various threads has been published by the National Cancer Institute.395 One HPV vaccine contains VLPs of HPV types 6, 11, 16, and 18. The type 16 and 18 serotypes are responsible for 70% of cervical cancers in the United States. A second, bivalent HPV vaccine was licensed in the United States in 2009. The quadrivalent vaccine is also effective against genital warts, whereas the bivalent targets only cervical cancer.

When first approved in 2006, the quadrivalent vaccine was for use in females only; by 2009 it became clear that boys also

developed cancers induced by HPV and needed to be vacci- nated as well, and approval was extended. A nine-valent recombinant HPV vaccine was approved in 2014 to be admin- istered to females ages 9 to 26 years and males ages 9 to 15 years.

The use of proteins, polysaccharides, and recombinant technology broadened the possibilities for vaccine develop- ment immensely but challenges remained. Some diseases, such as ***N. meningitidis serogroup B*** had eluded vaccine devel- opment via traditional methods, though not for lack of trying. Meningococcus serogroup B did not have readily obvious pro- tective proteins, polysaccharides or antigens. Its capsular poly- saccharide was only weakly immunogenic and was thought to cause an autoimmune response.

In 1995 the first complete **genome sequence** was pub- lished (*H. influenzae*) and it immediately changed the game in vaccine research.396 In the decade that followed, more than 100 other pathogen sequences were completed and hundreds more undertaken. **DNA microarray** of these genomes allows for vastly more rapid identification of proteins that could be possible vaccine candidates.397

Meningococcus serogroup B is the first disease where genome sequencing has been successful in identifying such vaccine candidates. This was accomplished with **reverse vac- cinology**, a technology first pioneered by Rino Rappuoli and colleagues in Siena.398,399 As the MC58 strain of meningococ- cus serogroup B was being sequenced, a computer program was used to predict 600 specific antigens as possible vaccine candidates. Of the 600 antigens, 350 (58%) proved workable and 28 (5%) ultimately induced bactericidal antibodies. The technique identified more viable candidates for a meningo- coccal serogroup B vaccine than had been considered or tested in the preceding 40 years.397,400

DNA array analysis and reverse vaccinology have been further aided by the introduction of **proteomics** which allows the identification of subsets of all proteins that are present at any particular point in the life of the bacteria, thus further shortening the time to finding viable vaccine candidates.397

The first meningococcal serogroup B vaccine was licensed in the United States in October 2014; it was based on a previ- ously well-known virulence factor, factor H binding protein, two variants of which are contained in the vaccine.401 The

second meningococcal serogroup B vaccine, containing four proteins selected from the 28 that induce bactericidal anti- body, was licensed in January 2015, and is the **first vaccine produced by genomics** (also called **reverse vaccinology**.)402 Both meningococcal serogroup B vaccines are the first licensed vaccines to be approved under the FDA’s FAST TRACK program, which was implemented in 2012.

The majority of vaccines now being developed use new technologies that seem to offer greater safety and more pos- sibilities. The focus on subunit (purified protein or polysac- charide), genetically engineered, or vectored antigens has been greatly enlarged by the addition of powerful new techniques such as DNA array analysis, reverse genetics and proteomics. However, older, classic methods such as attenuation and inac- tivation of whole virus continue to yield new vaccines, as the zoster vaccine demonstrates.

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As we continue in the second decade of the 21st century, the prospect for vaccines is stellar. Since 2000, 13 important vaccines have been licensed in the United States and Europe: a heptavalent pneumococcal conjugate vaccine (2000); a cold-adapted influenza vaccine (2003); a meningococcal quadrivalent conjugated polysaccharide vaccine (2005); a high-potency zoster vaccine (2006); the quadrivalent HPV VLP vaccine (2006); a pentavalent rotavirus reassortant vaccine (2006); an attenuated human strain rotavirus vaccine (2008), a second-generation, Vero-cell–produced, inactivated JE vaccine (2009); a bivalent HPV vaccine (2009); a 13-valent pneumococcal conjugate vaccine (2010); a nine-valent HPV vaccine (2014); and, at long last, two meningococcal sero- group B vaccines (2014, 2015). Three cholera vaccines, two killed and one live, have also been licensed in other parts of the world.

This chapter has chronicled the remarkable impact of vac-

cination on the health of the world’s population. In the 21st century, that impact will continue to increase, as a new genera- tion of scientists create, innovate, and plumb the depths of genetic engineering, genomics and the vast array of technolo- gies they have spawned. Despite the vicissitudes of the natural and the political world, the new technologies augur well that the platinum age of vaccinology is indeed upon us.

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