**Louis Pasteur by Albert Edelfelt, oil on canvas, 1885.** Wikimedia Commons

# Remembering Pasteur on the bicentennial of his birth

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December 27, 2022 marked the bicentennial of Louis Pasteur's birth (1822-1895). Pasteur was a distinguished French scientist whose discoveries had a great impact on medicine, especially in preventing infectious diseases through vaccination.

Pasteur's pioneering work on vaccination takes on added significance since the onset of the COVID-19 pandemic. The monumental achievement of producing a COVID-19 vaccine was made possible by the brilliant work of many scientists and researchers, but some credit needs to go to Louis Pasteur who paved the way.

## Pasteur the scientist

Pasteur was born the third child to a family of modest means.[1] An average student in primary school, he passed and earned his Bachelor of Science and Mathematics degree in 1842, with a mediocre grade in chemistry. He took a year off to prepare for the entrance exam for *École Normale Supérieure*, passed with high marks, and successfully graduated in 1845. He was recruited by Antoine J. Balard (1802-1876), a noted chemist, to work in his laboratory.[1]

Pasteur was extremely dedicated to his research, a fact accepted by his wife, Marie, whom he married in 1849. She encouraged his every endeavor and served as his most trusted confidant.[1]

Pasteur was a keen observer, often noticing details overlooked by other researchers. In 1848 he detected and separated the left and right-handed crystals of the tartaric acid enantiomers, opening up the field of stereochemistry.[2]

He was a methodical worker, but he also had an innovative approach that allowed him to devise ingenious ways of exploring an issue. A major argument about the source of life existed in 1800s. An important hypothesis, laid out by Aristotle in his book *History of Animals*,[3] held that life springs spontaneously from certain conditions—air, water, soil, and light. This theory remained popular into the 1800s, although a growing number of scientific minded thinkers disagreed. It was Pasteur's experiments with the swan neck flask that finally and emphatically ended the argument. The swan neck shape sealed the sterilized broth in the flask, preventing contact with ambient air and microorganisms. Only when the flasks were opened did life emerge[1,4]

Pasteur often focused on a problem for long periods of time, stretching into years, until he was satisfied that he had answered a scientific question and had thoroughly eliminated any confounding variable. It took him five years, to develop his rabies vaccine.

## The germ theory of disease

Pasteur, along with the German physician Robert Koch (1843-1910), were pioneers of the germ theory of disease. Pasteur famously commented that to unravel the cause of an infectious disease "seek the microbe."[1]

The demonstration of isomers of crystals, some argue his greatest triumph, generated considerable attention for Pasteur. In 1856, one of his students requested help for a

problem his father was experiencing with fermentation of beetroot, producing a sour product.[4] Pasteur's attention to fermentation led to the recognition that some microorganisms produce alcohol whereas others produce lactic acid (souring). This field of interest led to the further recognition that the souring or spoilage of many foods was due to microorganisms, and furthermore, that by heating the fluid or food, the microorganisms could be destroyed and the fermentation delayed.[1,4] This was the birth of pasteurization, a process with major benefit, especially noted in the dairy industry, where pasteurization of milk is now the standard. Pasteurization during the 1860s helped save the French wine export industry from product souring caused by *mycoderma aceti*.[1,4] Pasteurization of milk and other food products has since helped to reduce the incidence of food borne infections in these consumer items.[5]

A corollary of this work was the attribution of many diseases to infection by microbes, also known as the germ theory of disease. Pasteur believed that by eliminating these pathogenic microorganisms, one could cure or prevent infectious diseases.[1]

The noted British surgeon, Joseph Lister (1827-1912) was influenced by Pasteur's ideas on germ theory, and pioneered surgical antisepsis in 1865.[1] Pasteur also advocated the use of aseptic technique during surgery, including flaming the surgical blade and sterilizing the surgical field, so as to minimize post-operative infections.[1]

Pasteur also saved France's silkworm industry from infectious diseases that had caused huge losses to breeders. Pasteur had never touched a silkworm before he became involved with the silkworm problem, but in five years of careful research (1865-1870), he distinguished the two infectious diseases responsible, pébrine and flacherie, and learned how to prevent them.[1,4]

During the 1870s, Pasteur began his studies on anthrax and avian cholera (*Pasteurella multocida*), which would lead to his developing successful vaccines against both bacteria. He would follow these accomplishments by developing a vaccine against rabies.

## Vaccinations

When Pasteur began to study infectious diseases, little was known about the microorganisms that caused infection, let alone the concept of prevention or the phenomenon of immunity. Smallpox vaccine, based on Edward Jenner's (1749-1823) cowpox vaccine, was widely recognized to protect from smallpox, but it was the only human vaccine in use at the time. How this technique prevented smallpox, however, was not understood.

One of Pasteur's oft-quoted remarks was, "Chance only favours the mind which is prepared."[1] This remark came true for Pasteur with a break in his research on avian cholera, which led to a vaccine against that disease.

In 1879, Pasteur instructed his assistant Charles Chamberland (1851-1908) to inoculate chickens with a fresh culture of avian cholera bacteria. Chamberland did not carry out this order but left for a holiday.[4] Upon his return, the month old bacterial culture, when injected into chickens, caused some symptoms of infection, but was no longer lethal.

Chamberland assumed an error was made, but Pasteur, guessed that the recovered chickens might now be immune to the disease. When Pasteur injected them with a lethal dose of avian cholera, the chickens remained well. Pasteur postulated that the virulence of avian cholera progressively diminished when exposed to air thus forming an attenuated strain of bacteria, that when injected into chickens, produced immunity to the disease. Although much has been made of the lucky break in the development of this vaccine, that accident came from many months of hard work in Pasteur's laboratory.

During 1881, Pasteur applied his immunization method to anthrax, using heat cultivation at around 42 degrees Centigrade to attenuate its infectivity to sheep, while preserving its immunogenic capabilities.[6] A dramatic public display of the effectiveness of the anthrax vaccine occurred at Pouilly-le-Fort, France. Pasteur vaccinated 24 sheep against anthrax while another 24 sheep served as controls. All the sheep then received lethal doses of anthrax. All vaccinated sheep survived, while all the unvaccinated sheep died.[7]

Pasteur was hailed in the public media for this remarkable scientific feat. Although Pasteur was an advocate of attenuated, but live microorganisms for immunization, the anthrax vaccine he used appears to have been a potassium dichromate inactivated vaccine.[4,6]

## Pasteur's rabies vaccine

In 1880, Pasteur turned his attention to rabies. Pasteur's assistants were Emile Roux (1853-1933), Charles Chamberland, and Louis Thuillier (1856-1883).

Rabies proved to be a great challenge. Working with rabid animals was risky. Furthermore, they were now dealing with an unknown microorganism, a pathogen they could not isolate or see under a microscope. Viruses were not as yet identified as being infectious agents different from bacteria. That discovery was made in 1892, with Dmitri Ivankovsky's (1864-1920) studies on tobacco

mosaic disease.[8] In 1898, Martinus Beijernick (1851-1931) called this non-bacterial pathogen a "virus."[8]

Pasteur was convinced that with rabies he was dealing with an infectious disease, despite not being able to culture the pathogen by the usual in vitro methods that successfully grew bacteria.[1]

To shorten rabies prolonged incubation period, Pasteur developed an in vivo culture method that used trephination to place nervous tissue taken from infected dogs beneath a rabbit's dura mater. By passing infected nervous tissue from rabbit to rabbit, Pasteur succeeded in shortening the incubation period for viral transmission to seven days ("fixed" virus) in contrast to the 15 day incubation period that occurred upon initial infection of the rabbits by "street virus" taken from rabid dogs.[9,10]

We now know that during serial passage in rabbits, spontaneous mutations occur in the rabies virus, selecting mutant variants that grow better than the parent virus. The resulting fixed virus causes more severe neuropathological changes in the rabbit brain but is also poorly neuro-invasive after peripheral inoculation (e.g., into a muscle).[9]

Pasteur used this fixed virus to develop a rabies vaccine by desiccation in a glass flask. A picture of the flask was painted by the Finnish artist Albert Edelfelt (1854-1905) in 1885, when he visited Pasteur.[1] It shows Pasteur contemplating the glass flask he used to desiccate suspended rabid spinal cord taken from rabbits. The ensuing desiccation denatured viral proteins, making the spinal cord non-infectious to rabbits after one to two weeks.[9,10]

To produce immunity against rabies, Pasteur subcutaneously inoculated dogs, first with 15 day dried rabid spinal cord. On succeeding days, he repeated this procedure, substituting spinal cord material desiccated for progressively shorter periods. The technique worked in dogs both on a pre-exposure basis and also after a bite from an infected animal (post-exposure prophylaxis).[10]

On July 6, 1885, the Pasteur rabies vaccine was administered to Joseph Meister, a nine-year-old boy bitten by a rabid dog.[10,11] This was Pasteur's first reported case for post-exposure prophylaxis in a human, and the boy survived. By April 1886, 726 persons had been vaccinated with only four rabies deaths, results that suggested that

**Louis Pasteur at his 70th birthday jubilee at the Sorbonne, December 27, 1892. This 1902 oil painting is by Jean-Andre Rixens (1846-1925) and shows Joseph Lister, with outstretched arms, greeting Pasteur in the grand amphitheater of the Sorbonne. Pasteur is accompanied by the President of the Republic, Sadi Carnot (1837-1894).** With permission of Institut Pasteur/Musee Pasteur

the vaccine was successful in reducing rabies transmission, as compared with historical controls.[9]

## Vaccine controversy

Pasteur's rabies vaccine received considerable publicity in the news media and soon persons bitten by rabid animals traveled from all over the world to Paris to receive the newly developed vaccine. A Pasteur Institute in Paris and Pasteur Institutes in other countries were established to help satisfy the growing demand for the rabies vaccine.

Among some members of the medical community the rabies vaccine was highly controversial as it included a live, albeit attenuated, virus, raising fears that it could potentially transmit the disease.[12] This concern was eased in 1911 by the introduction of the Semple phenol inactivated rabies nerve tissue vaccine. Unfortunately, neuro-paralytic reactions could occur from the Semple vaccine due to an immune response to myelin basic protein in the vaccine's nerve tissue. The rabies vaccine therefore underwent several modifications to become its well tolerated modern day versions (human diploid cell vaccine, purified chicken embryo cell vaccine and the Purified Vero cell rabies vaccine).[9]

Pasteur's success with the rabies vaccine, as well as his work on other vaccines, spurred others to identify vaccines for many other diseases. Today, the World Health Organization lists more than 20 diseases that can be prevented by vaccination, including COVID-19.[13]

As of today, more than 5.51 billion people worldwide have received a COVID-19 vaccine.[14] More work needs to be done to make the vaccine available to the rest of humanity and to thus slow the development of viral variants that might prove to be more infectious and dangerous than the current strains of COVID-19 virus.

## A foundation for presentation

Pasteur's multiple discoveries laid much of the foundation for the prevention of infectious diseases, both via pasteurization and vaccination, and also through his mentorship of future scientists. He developed the Pasteur Institute, initially to refine and produce rabies vaccines. That institute now includes 33 laboratories within the Pasteur International Network.[15] While Pasteur never received the Nobel prize, which was introduced five years after his death, there have been 10 Nobel Laureates from the Pasteur Institute.[16]

In 1892, Pasteur was honored at the Paris-Sorbonne, University on the occasion of his 70th birthday. In attendance was Joseph Lister who embraced Pasteur in a show of honor and admiration. In his speech, Pasteur stated his belief that, "…Science and Peace will triumph over ignorance and war, that nations will unite, not to destroy but to build, and that the future will belong to those who will have done most for suffering humanity."[1]

**Reference**

1. Vallery-Radot R. The life of Pasteur. New York: The Sun Dial Press, Inc; 1937.
2. Flack HD. Louis Pasteur's discovery of molecular chirality and spontaneous resolution in 1848, together with a complete review of his crystallographic and chemical work. Acta Cryst. 2009; A65 (Pt. 5): 371-89.
3. Aristotle. History of Animals. DW Thompson, trans. The Internet Classics Archives. http://classics.mit.edu/Aristotle/history_anim.2.ii.html.
4. Ullman A. Louis Pasteur: French chemist and microbiologist. Britannica Online. https://www.britannica.com/biography/Louis-Pasteur.
5. Pasteurization: heating process. Britannica Online. https://www.britannica.com/technology/pasteurization.
6. Smith KA. Wanted, an anthrax vaccine: dead or alive? Med Immunol. 2005; 4(1): 5.
7. Pasteur L, Chamberland C, Roux E. Summary report of the experiments conducted at Pouilly-le-Fort, near Melun, on the anthrax vaccination. Yale J Biol Med. 2002; 75(1): 59-62.
8. Tobacco Mosaic Virus. Britannica Online. https://www.britannica.com/science/tobacco-mosaic-virus.
9. Hoenig LJ, Jackson AC, Dickinson GM. The early use of Pasteur's rabies vaccine in the United States. Vaccine. 2018; 36(30): 4578-81.
10. Pasteur L. Méthode pour prévenir la rage après morsure. C R Acad Sci. 1885; 101: 765-74.
11. Berche P. Louis Pasteur, from crystals of life to vaccination. Clin Microbiol Infect. 2012; 18 (Suppl. 5): 1-6
12. Hoenig LJ. Triumph and controversy: Pasteur's preventive treatment of rabies as reported in JAMA. Arch Neurol. 1986; 43(4): 397-9.
13. World Health Organization. Immunization, Vaccines and Biologicals. https://www.who.int/teams/immunization-vaccines-and-biologicals.
14. Holder J. Tracking Coronavirus Vaccinations Around the World. The New York Times. January 8, 2023. https://www.nytimes.com/interactive/2021/world/covid-vaccinations-tracker.html.
15. Institut Pasteur. Pasteur Foundation. https://pasteurfoundation.org/about/institut- pasteur/.
16. The Nobel Prize. Pasteur Foundation. https://pasteurfoundation.org/research/nobel-prize-winners/.

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