

MILESTONE 3

The first live attenuated vaccines

Awareness of Edward Jenner's pioneering studies of smallpox vaccination (MILESTONE 2) led Louis Pasteur (1822–1895) to propose that vaccines could be found for all virulent diseases.

Pasteur began to study chicken cholera in 1877 and by the following year had succeeded in culturing the causative organism, *Pasteurella multocida*. In 1879, Pasteur discovered by chance that cultures of this bacterium gradually lost their virulence over time. Before leaving to go on a holiday, Pasteur had instructed an assistant to inject the latest batch of chickens with fresh cultures of *P. multocida*. The assistant forgot to do this, however, and then himself went on holiday. On his return, Pasteur's assistant inoculated the chickens with the cultures, which by this time had been left in the laboratory for a month, stoppered only with a cotton-wool plug. The inoculated chickens developed mild symptoms but recovered fully.

Another scientist might have concluded that the cultures had (mostly) died, but Pasteur was intrigued. He injected the recovered chickens with freshly cultured cholera bacteria. When the birds remained healthy, Pasteur reasoned that exposure to oxygen had caused the loss of virulence. He found that sealed bacterial cultures maintained their virulence, whereas those exposed to air for differing periods of time before inoculation showed a predictable decline in virulence. He named this progressive loss of virulence 'attenuation', a term still in use today.

Pasteur, along with Charles Chamberland and Emile Roux, went on to develop a live attenuated vaccine for anthrax. Unlike cultures of the chicken cholera bacterium, *Bacillus anthracis* cultures exposed to air readily formed spores that remained highly virulent irrespective of culture duration; indeed, Pasteur reported that anthrax spores isolated from soil where animals that died of anthrax had been buried 12 years previously remained as virulent as fresh cultures. However, Pasteur discovered that anthrax cultures would grow readily at a temperature of 42–43 °C but were then unable to form spores. These non-sporulating cultures could be maintained at 42–43 °C for 4–6 weeks but exhibited a marked decline in virulence over this period when inoculated into animals.

Accordingly, in public experiments at Pouilly-le-Fort, France, conducted under a media spotlight reminiscent of that on today's

COVID-19 treatment trials, 24 sheep, 1 goat and 6 cows were inoculated twice with Pasteur's anthrax vaccine, on 5 and 17 May 1881. A control group of 24 sheep, 1 goat and 4 cows remained unvaccinated. On 31 May all the animals were inoculated with freshly isolated anthrax bacilli, and the results were examined on 2 June. All vaccinated animals



Louis Pasteur holding rabbits, which were used to help develop the vaccine for rabies. Credit: World History Archive / Alamy Stock Photo

remained healthy. The unvaccinated sheep and goats had all died by the end of the day, and all the unvaccinated cows were showing anthrax symptoms. Chamberland's private laboratory notebooks, however, showed that the anthrax vaccine used in these public experiments had actually been attenuated by potassium dichromate, using a process similar to that developed by Pasteur's competitor, Jean Joseph Henri Toussaint.

In 1881, Victor Galtier (who had already demonstrated transmission of rabies from dogs to rabbits) reported that sheep injected with saliva from rabid dogs were protected from subsequent inoculations. These surprising observations piqued Pasteur's interest and he went on to develop the first live attenuated rabies vaccine.

Despite failing to culture the rabies-causing organism outside animal hosts or to view it under a microscope (because, unknown to Pasteur, rabies is caused by a virus rather than a bacterium), Pasteur discovered that the virulence of his rabies stocks, maintained by serial intracranial passage in dogs, decreased when the infected material was injected into different species. Starting with a highly virulent rabies strain serially passaged many times in rabbits, Pasteur air-dried sections of infected rabbit spinal cord to weaken the virus through oxygen exposure, as explained in Pasteur's 26 October 1885 report to the French Academy of Science. All 50 dogs vaccinated with this material by Pasteur were successfully protected from rabies infection, although we now understand attenuation to result from viral passage through dissimilar species, rather than air exposure.

Up to this point, however, Pasteur had no proof that his vaccines, a term coined by Pasteur to honour Jenner's work, would be effective in humans. Reluctantly — as Pasteur was not a licensed physician and could have been prosecuted for doing so — on 6 July 1885, Pasteur used his rabies vaccine, in the presence of two local doctors, to treat 9-year-old Joseph Meister, who had been severely bitten by a neighbour's rabid dog. Joseph Meister received a total of 13 inoculations over a period of 11 days, and survived in good health. Pasteur's reluctance might also be accounted for by posthumous analysis of his laboratory notebooks, which revealed that Pasteur had vaccinated two other individuals before Meister; one remained well but might not actually have been exposed, and the other developed rabies and died.

By the end of 1885, several more desperate rabies-exposed people had travelled to Pasteur's laboratory to be vaccinated. During 1886, Pasteur treated 350 people with his rabies vaccine, of whom only one developed rabies. The startling success of these vaccines led directly to the founding of the first Pasteur Institute in 1888.

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ORIGINAL ARTICLES Pasteur, L. An address on vaccination in relation to chicken cholera and splenic fever. *Br. Med. J.* 2, 283–284 (1881) | Pasteur, L. Méthode pour prévenir la rage après morsure. *C. R. Séances Acad. Sci.* (Séance du lundi 26 octobre 1885)

FURTHER READING Jackson, A. C. (ed.) *Rabies: Scientific Basis of the Disease and Its Management* (Academic Press, 2013)