

# Louis Pasteur, from crystals of life to vaccination

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## Abstract

Louis Pasteur (1822–1895) is an exceptional scientist who opened a new era in medicine and biology. Starting from studies on crystals of by-products of wine fermentation, he first defined a distinct chemistry between dead and living matters. He then showed the role of living microbes in the fermentation and putrefaction processes. This brought him to challenge the two-millennium-old theory of spontaneous generation, using remarkably well-designed experiments. His observations on epidemics in silkworms allowed him to demonstrate the role of specific germs in infectious diseases. His discovery of the vaccine against fowl cholera can be considered as the birth of immunology. Finally, he became universally recognized through his famous vaccinations against anthrax and rabies.

**Keywords:** Anthrax, fermentation, germ theory, infectious diseases, Louis Pasteur, rabies, Robert Koch, spontaneous generation, vaccination

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Louis Pasteur is probably one of the most famous scientists in the world, universally recognized as a benefactor of Humanity [1–4]. His name is associated with many new concepts and discoveries, such as the crystals of life, the theory of germs, and vaccination. Apparently, nothing predestined this man of humble origin to become a giant in the field of medicine and biology, to such a point that he opened up a new era and saved countless lives. Indeed, he came into the world during an exciting and tumultuous period of history, following the French Revolution, a time of great discoveries in science and medicine. Pasteur was born in December 1822, in the small city of Dole situated in the eastern region of France. His father, who had served as a sergeant in Napoleon's army, opened a tannery on his return home. As a schoolboy, Pasteur was a serious, not particularly brilliant child, but diligent and wishing to fulfil his father's dream, namely to become a school teacher! Although he was a talented painter and artist, he decided to pursue studies in scientific disciplines (Fig. 1).

## Crystals of life 1847

In 1843, Louis Pasteur was admitted, through a highly competitive examination, to the *Ecole Normale Supérieure* in Paris, a training school for the professors of colleges. There, he specialized in the physics and chemistry departments and, as a chemist, became interested in the origins of life. Pasteur decided to work in the field of crystals to achieve his doctorate. While still young, he accomplished a great feat in working on the optical activity of crystals in response to polarized light, using tartaric acid and tartrates derived from fermentation. Wine fermentation is known to generate 'tartar', by-products mainly constituted by large crystals of tartaric acid. These crystals are optically active and rotate polarized light. Smaller crystals, needle-like tufts, are sometimes present, as another form of tartaric acid, designated as paratartratic acid or racemic. According to the German chemist Eilhard Mitscherlich, these crystals are optically inactive, although they display the same chemical composition, shape and refraction. For Pasteur, this was impossible. He became convinced that these components must exhibit some chemical differences, which might be expressed in the crystals' shape. He therefore prepared crystals of 19 different salts of tartrates and paratartrates, which he examined under the microscope. He detected small facets with differ-



**FIG. 1** The young Louis Pasteur, the 'Angel of Science'.

ent orientation according to the crystals. The facets in each of the tartrate salts had the same orientation. In contrast, some facets of each paratartrate salt were oriented in one direction but others were in the opposite direction: there was a mixture of crystals. He manually separated the right and the left crystals, which were identical in shape to crystals of tartrate salts. The optical inactivity to rotate the polarized light was therefore explained by the mixture of paratartrate crystals. He presented his research thesis in 1847.

Pasteur made his first step toward biology by chance, several years later, while working in Strasbourg in 1857. During the summer, he observed that certain fungi grew readily in solutions of calcium paratartrate, which were composed equally of left and right crystals. He found that the contaminated solutions became optically active with time, as the right component was consumed by fungi. This was a new way of separating paratartrate crystals! For Pasteur, only living agents could produce optically active asymmetric compounds. This was the only sharp defined difference between the chemistry of dead and living matter [5].

### **The end of the theory of the spontaneous generation 1857–1873**

Pasteur became progressively interested in the process of fermentation that produces the crystals of life. What was the nature of fermentation? At that time, the prevailing paradigm

was that fermentation was a chemical catalytic process due to 'ferments'. Naturalists described under the microscope the presence of numerous yeasts always accompanying the alcoholic fermentation. It was largely believed that these yeasts were nothing but a complex chemical substance, acting as a catalyst in the conversion of sugars into alcohol. Various other 'ferments' (e.g. lactic acid, acetic acid) were also considered as complex material acting as catalyst, purely by chemical contact. This view was strongly supported by most eminent chemists, such as Justus von Liebig, Jöns Berzelius and Friedrich Wöhler. However, an alternative hypothesis emerged, claiming that ferments were living microbes. In 1835, Charles Cagniard-Latour, followed by Theodor Schwann and Friedrich Kützing in 1837, demonstrated that fermentation started with the presence of yeasts, progressed with their multiplication, and stopped when yeast growth was over [6,7]. These authors concluded that yeasts were living cells multiplying during the fermentation process. In addition, heating and antiseptics, for example heavy metals such as arsenic, stopped the process of fermentation. The hypothesis claiming that fermentation was a living process was rejected by most chemists.

While working in Lille, Pasteur became involved in this controversy (Fig. 2). He was approached by an industrialist who had encountered difficulties with the production of alcohol by the fermentation of beer. In many cases, alcohol was contaminated with undesirable substances during the course of fermentation for unknown reasons. Pasteur performed many tests in the factory, trying to understand the cause of beer 'disorders'. Under the microscope, he observed the presence of small globules in the fermenting juice, associated with fine structures smaller than yeasts, which turned out to be bacteria. With his polarimeter, he also found that the fermented juice was optically active. He also succeeded in isolating an optically active component, identified as amyl alcohol. This substance clearly could not derive from the degradation of ethyl alcohol. Pasteur was then convinced that the process of fermentation was caused by the activity of living ferments: 'The chemical act of fermentation is essentially a phenomenon correlative with a vital act, beginning and ending with the latter. I believe that there is never any alcoholic fermentation without simultaneously the organization, development, multiplication of the globules, or the pursued, continued life of globules which are already formed'. In 1857, he published a work on lactic fermentation that can be considered as the landmark of future germ theory [8]. From 1857 to 1873 he studied many fermentations, such as those of sour milk, wine, beer and vinegar. He concluded that all fermentation processes are due to specific living ferments (bacteria or yeasts). Wine and beer



FIG. 2 Louis Pasteur, at the time of his studies on fermentation.

disorders were caused by contaminant bacteria [9,10]. He discovered life without oxygen (anaerobic life of the butyric fermentation) and proposed a prophylactic treatment of diseases: iterative heating or pasteurization [11]. These findings led him to study the hypothesis of spontaneous generation.

Since Aristotle (sixth century BC), it had been generally believed that the metamorphosis and decomposition phenomena, such as decay, putrefaction, rotting, fermentation and mouldering, resulted from a 'vital force' existing within the organic substances. Many living things came forth from non-living matters because the non-living material contained *pneuma* or 'vital heat'. This theory of the spontaneous generation of living creatures was still prevailing in Pasteur's time, despite remarkable experimental and premonitory works by the Italians Francesco Redi (1626–1697) and Lazzaro Spallanzani (1729–1799) [12]. Using well-designed experiments, Spallanzani had produced evidence in 1765 for the preventive role of heating on broth infusions, suggesting that the air was a source of contamination of the culture broth. Pasteur reproduced these experiments using yeast infusions (1861–1865). These data were challenged by the naturalist and biologist Felix Archimède Pouchet (1800–1872), who failed to reproduce Pasteur's results using hay infusions. This was the origin of a heated controversy before the French *Académie des Sciences* [12–14]. Pasteur designed wonderful experiments with heating to support his results. He even succeeded in preserving sterility without heating using

swan-neck flasks and cotton filters: indeed, he brought solid evidence that the air contains microbes that contaminate broth cultures. This marked the end of the two-millennium-old theory of spontaneous generation.

## The germ theory of infectious diseases

Since Antiquity, the origin of epidemics had remained mysterious. It was generally accepted that these phenomena were related to miasmas, involving the influence of weather and planets. During the nineteenth century, several observations pointed out the role of germs as a possible cause of epidemics. First, work by the Italian Agostino Bassi published in 1835 on the 'white muscardine', an epidemic disease of domesticated silkworms (*mal del segno*), which became progressively covered by a cotton-like duvet. Bassi observed under the microscope that a fungus (*Botrytis bassiana*) multiplied in and devoured the silkworms' bodies. He concluded that the fungus was the cause of the disease. He efficiently used a prophylaxis based on hygiene measures and phenol solution to prevent the fungal spread. A few years later in 1845–1847, another fungus was stigmatized as the agent of 'Potato Blight', a terrible epidemic of crops in Ireland. Following several rainy summers in Ireland the potato crops were entirely destroyed. As this was the only food source for the population, the 'Great Irish Famine' occurred with 1 million dead of starvation and 1.5 million migrating, out of a population of 8 million. In 1846, the Reverend Miles J. Berkeley observed that the potato tubers and leaves were massively invaded by a microscopic fungus, termed *Phytophthora infestans*. In an article entitled 'Observations, Botanical and Physiological, on the Potato Murrain', he assumed that this fungus was responsible for the destruction of crops. This hypothesis was experimentally proven in 1863 by the German botanist Heinrich Anton de Barry (1831–1888), who inoculated spores of *P. infestans* on healthy potato leaves and observed the subsequent growth of mycelium and the appearance of the characteristic black spots of potato blight [12].

There were also early observations in patients that suggested the role of fungi in communicable diseases. In 1839, the dermatologist Johann Schoenlein showed that *tinea favosa*, a cutaneous mycosis widely spread in the population, was caused by *Trichophyton schoenleinii*. Soon afterwards, Theodor Berg, David Gruby and K.F. Eichstedt demonstrated the role of specific fungi in various mycoses, including thrush (candidiasis), *tinea* and *pityriasis versicolor* [12].

In 1847, Ignaz Semmelweis (1818–1865), a young medical doctor working at the Vienna General Hospital,

noticed that the mortality from childbed fever was higher in the maternity clinic of Prof. Johann Klein (18%), compared with that of the nearby maternity clinic run by Prof. Franz Bartsch (3%). He suspected that puerperal fever could be transmitted by the medical students performing autopsies: 'The causation I search is inside our clinic: it is on the fingers of students'. He prevented the spreading of invisible particles by hand washings with chlorinated lime, so successfully reducing mortality. He summarized his work in 1861 in a famous book entitled *Etiology, Concept and Prophylaxis of Childbed Fever* [15,16].

Pasteur became involved in the field of infectious diseases through an epidemic in silkworms in the south of France (1865–1870). In 1853, a mysterious disease, called 'pébrine', began to attack the French silkworm nurseries ('magnaneries'). Silkworm larvae, which normally feed on mulberry leaves, became covered in brown dots, like pepper seeds, and were unable to spin silkworm thread. By 1865, the disease spread to most silkworm-producing areas, and the industry was nearly ruined in France, as in the rest of western Europe. A closely related form of this disease, known as 'flacherie' (variants are also known as 'morts-flats', 'gattine') was also described. *Flacherie* (or 'flaccidness') was characterized by dark brown silkworms. The Academician Jean-Baptiste Dumas asked Pasteur to work on this disease. He went to Alès, a small city in the south of France, in the heart of the silkworm district in the Cevennes. He studied this disease from 1865 to 1870 and established that there were two entirely different diseases, previously considered as variants of the same disease. One was 'pébrine' caused by the parasite protozoan (*Nosema bombycis*), and the other was *flacherie*, which was believed by Pasteur to be primarily nutritional in origin, with bacterial intestinal proliferation. It is known today that *flacherie* is the result of a virus with intestinal bacterial superinfection. He proposed an effective prophylaxis based on the selection of non-infected eggs by microscopic examination [1–4]. Then, Pasteur proposed the germ theory to explain all infectious diseases. A Scottish surgeon, Joseph Lister (1827–1912), reading Pasteur's works, was convinced that suppurated wounds and gangrene were the result of contaminant bacteria. In 1867, he confirmed Pasteur's conclusions with his own experiments using antiseptics such as phenol to successfully treat wounds.

The germ theory had important consequences. First, it triggered a hunt for pathogens: within 20 years, the bacteria responsible for most infectious diseases were discovered, such as leprosy, tuberculosis, diphtheria, cholera, bubonic plague, following which new methods to prevent spread of epidemics appeared, including hygiene, pasteurization and vaccines.

## Discovery of vaccines 1877–1895

The discovery by Louis Pasteur of the vaccine against fowl cholera can be considered as the birth of immunology [17]. This endemic disease spread via devastating epidemics, rapidly destroying the breeding of chickens. Thousands of chickens, packed into hen houses, were dying of diarrhoeal disease within 48 h. In 1878, Pasteur succeeded in culturing the causative agent of fowl cholera, a highly virulent bacterium, *Pasteurella multocida*, and the disease was easily reproduced by inoculation. In 1879, Pasteur observed by chance that 'old' cultures lost their virulence. Chickens inoculated with old cultures became protected against a virulent wild strain and survived. Interestingly, the few chickens surviving the disease still excreted virulent bacteria, so indicating the existence of healthy carriers, an important concept to explain the mysterious spread of germs during epidemics. In February 1880, Pasteur presented his results to the French *Académie des Sciences*, in a memorandum entitled '*Sur les maladies virulentes et en particulier sur la maladie appelée vulgairement choléra des poules*' ('On virulent diseases and particularly on the disease commonly called fowl cholera') [18]. He coined the phenomenon 'vaccination', in honour of Edward Jenner (1749–1823), who had performed the first inoculation of cowpox to prevent smallpox in 1796.

Pasteur then became interested in the prevention of anthrax, a widely spread plague of cattle [19,20]. In 1874, a rural physician, Robert Koch (1843–1910), demonstrated the role of *Bacillus anthracis* in this disease, and succeeded in preserving its pathogenicity after eight subcultures. He also discovered the resistant spores of this bacterium. Pasteur confirmed its causative role by subculturing this bacterium one hundred times, without altering the high virulence of the pathogen. Then, he tried in 1881 to attenuate its virulence by preventing spore formation. This was achieved by maintaining live bacilli in the presence of oxygen. In optimal conditions, live bacilli were grown for 8 days in ventilated culture broth incubated at 42–43°C. Bacilli progressively lose their virulence, becoming harmless in animals, such as rabbits, sheep and guinea-pigs. The protocol of vaccination was more effective when inoculating an attenuated culture, followed 12 days later by a more virulent culture (March 1881). Pasteur finally used another attenuation procedure with potassium bichromate, followed by three passages in mice to reinforce the attenuated strain, according to a protocol by Jean-Joseph Toussaint. Then, Hippolyte Rossignol, a veterinarian from Melun, asked Pasteur to perform public experiments in the field. He accepted. A risky demonstration of the efficacy of vaccine! On 5 May 1881 at Pouilly-le-Fort,

a small village near Paris, he inoculated an attenuated culture into 24 sheep, one goat and six cows. On 17 May he injected a more virulent culture. Vaccinated animals and a control group were challenged on 31 May with a highly virulent culture. On 2–3 June, all sheep and the goat from the control group died, and the cows, which had received the same challenge, were very ill. In contrast, all the vaccinated animals survived in good health. The triumph of Pasteur was acclaimed by an enormous crowd and many reporters. In 1882, 85 000 cattle were vaccinated and in 1894 almost 3.4 million, so reducing anthrax mortality to 0.3% of the French livestock.

Shortly afterwards, Pasteur was called to study a disastrous epidemic of swine erysipelas in the Vaucluse, in the south of France. In March 1883 he identified the culprit as a highly virulent bacterium, now called *Erysipelothrix rhusiopathiae*. He then designed a new vaccine against this disease, by iterative passages through rabbits, an unrelated resistant animal species. This attenuated vaccine was then widely used. From 1886 to 1892, more than 100 000 pigs were vaccinated in France.

Vaccination against rabies in 1885 was the last discovery by Louis Pasteur that immortalized him (Fig. 3). Pasteur was 63 years old, handicapped by a permanent paralysis of his left arm as the sequel of a cerebral haemorrhage. In 1879, Pierre Galtier, a French veterinarian working in Lyon, demonstrated that rabies could be transmitted to rabbits by dog saliva. In 1880, Pasteur started studying rabies and succeeded in transmitting the disease to rabbits by intracerebral inoculation of brain extracts. The disease occurred after 1–2 weeks, instead of several weeks or months following bites. He found that virulence was exacerbated through iterative passages on rabbit brains shortening the incubation time to 6 days, with a rapidly fatal disease in dogs. This virulent strain was called 'fixed virus'. In 1884, Pasteur successfully attenuated the virulence of fixed virus by passages from dogs to monkeys, so increasing the incubation time for dogs, rabbits and guinea-pigs. Animals receiving the monkey virus were protected against the highly virulent fixed virus. Taking advantage of the long period of incubation of rabies, he then thought to use the attenuated vaccine as a therapy to stimulate the resistance of patients, i.e. the immune response. In 1885, with Emile Roux, Pasteur designed a new process to attenuate the fixed virus. Rabbit spinal cords infected with the fixed virus were desiccated and long-exposed to air in glass flasks. In these conditions, the virus lost its virulence in 15 days. By daily injections of rabbit spinal cord emulsions, progressively less and less attenuated, he could protect dogs against the highly virulent virus, including those inoculated by the intracerebral route [18–20].



FIG. 3 Louis Pasteur, at the time of the vaccine against rabies.

Then, Fate knocked at Pasteur's door. On 6 July 1885, Joseph Meister, a 9-year-old boy, came from Alsace to consult Dr Joseph Grancher at the *Hôpital des Enfants-malades* in Paris. He had been severely bitten by a rabid dog at the hands, legs and hips. The physician convinced a reluctant Pasteur to vaccinate the boy because he was inevitably going to die: it was the only way for him to stand a chance of survival! The treatment started on 7 July, 60 h after the accident. The boy received 12 consecutive injections of extracts of rabbit spinal cords attenuated by a 14-days desiccation procedure. On 16 July 1885, he was inoculated with a spinal cord extract from a rabbit dead of infection induced by the fixed virus. Joseph Meister survived; Pasteur had performed the first therapeutic vaccination to cure a universally lethal disease. In October 1885, Jean-Baptiste Jupille, a 15-year-old shepherd from the Jura, in the east of France, was severely bitten by a rabid dog. He too was successfully protected by vaccination. In October 1886, more than 2490 patients were vaccinated at the Pasteur laboratory, in Paris. Only ten died out of 1726 French patients [1,2]. In the same time, an investigation revealed mortality of about 40% among 320 unvaccinated patients bitten by rabid animals. After vaccination, mortality fell to 0.5% [19–21]. This universal triumph of anti-rabies vaccination contributed to the creation of the Pasteur Institute in 1888. Louis Pasteur set three objectives for the new Institute: 'It must be a public dispensary to treat rabies, a research

centre for infectious diseases, and a teaching centre for studies on microbiology'. This exceptional scientist, observer, skilful experimenter, and imaginative philosopher died in 1895, after opening a new era in medicine and biology.

### Conflict of interests

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There were no conflicting interests and no funding.

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