

### **Louis PASTEUR** **was a pioneer of crystallography, bacteriology,** **industrial microbiology, immunology.** **To the 200<sup>th</sup> anniversary of his birth**



*Portrait of L. Pasteur of Albert Edelfelt's work*

The name of Louis Pasteur, a world-renowned microbiologist, is now known to every educated person. His experiments that led to the discovery of the world of microbes and their role in our lives are described in many textbooks and reference books. For biologists it is interesting to know about this man as a person, his life path, beliefs, and worldview.

Louis Pasteur was born on December 27, 1822, in the Doli town in the family of a master tanner; five years later the family moved to Arbois, where he finished primary school. On the

advice of his teachers, who noted his abilities, he continued his education at the Royal College at Besançon, where he received his Bachelor of Letters degree in 1840 and Bachelor of Science in Mathematics degree in 1841). His next goal was to graduate from the École Normale Supérieure in Paris. In 1843 L. Pasteur was accepted to this institution, but the education there was expensive, so he simultaneously worked as an assistant teacher and supervisor, and then as a preparator in the chemical laboratory of Professor Balard. He also attended lectures by French chemist Jean-Baptiste-André Dumas and later became his assistant. Pasteur received his Master of Science degree in 1845, and two years later he received a doctorate in physics.

After graduating from the École Normale Supérieure, L. Pasteur was appointed professor of physics at the Dijon Lycée (secondary school). But there he had no laboratory where he could conduct experiments. Under Professor Balard's patronage, he received an appointment as a professor of chemistry for the Faculty of Natural Sciences at the University of Strasbourg. There he met Marie Aimée Laurent, the daughter of the University's rector. The couple married in 1849. g. They had five children, but three of

them died of typhoid fever in childhood. Son Jean-Baptiste Pasteur received a law degree and worked as a diplomat in Rome, Copenhagen, Madrid, and Athens.

**Scientific activity of L. Pasteur in stereochemistry.** During his studies at the École Normale Supérieure, his attention was focused on crystallography, and his research in this area became the topic of his diploma work. He continued these studies after graduation. When studying the optical properties of tartrates and paratartrates, he found that they showed right and left asymmetry, but a balanced mixture of right and left crystals was optically inactive. L. Pasteur hypothesized that this phenomenon is due to molecular asymmetry. According to biographers, he laid the foundations of stereochemistry with his experiments and conclusions. Over the next 10 years, L. Pasteur continued to study the ability of organic substances to rotate the plane of polarized light and the relationship between the crystal structure and molecular configuration.

**Research in the field of industrial microbiology.** In 1854, L. Pasteur was appointed dean and professor of chemistry at the new university in Lille. He was approached by winemakers with a request to solve the problem of wine souring. This led L. Pasteur to start researching the fermentation process. Then the 35-year-old chemistry professor began to study the world of microbes, which was absolutely unknown to him, and made extremely important discoveries in this area. The wine and beer industry in France suffered from souring of products due to the contamination during export. At the request of Emperor Napoleon III, L. Pasteur investigated the contamination of wine and showed that it was caused by microbes. As part of these works, he made a discovery unexpected for himself and the scientific community that yeast is responsible for alcoholic fermentation, and certain bacteria cause the souring of wine. L. Pasteur continued his research in Paris where he was offered in 1857 the position of manager

and head of scientific research at the École Normale Supérieure.

Now all over the world, this process is known as pasteurization. Today, pasteurization is almost not used in winemaking, because this process kills microorganisms that are useful for obtaining aged wines. Pasteurization is now applied to many foods and beverages, including milk. After Pasteur's success with wine, he focused his research on beer and developed the basic technological processes of brewing.

**Pasteur effect.** L. Pasteur expanded his research, paying attention to butyric acid fermentation. The results of these works led him to the discovery that the fermentation process can be stopped by aeration of the fermentation liquid. This phenomenon is now called the «Pasteur effect». Based on the observed effect, he concluded that it is due to the presence of a life form that can function only in the absence of oxygen. L. Pasteur introduced the terms «aerobic» and «anaerobic» into microbiology to refer to organisms that live in the presence or absence of oxygen, respectively.

**Microbes or self-generation.** Fermentation studies strengthened Pasteur's belief in the crucial role of microorganisms in many processes, although such an opinion was rather exceptional, contrary to the then existing theory, which claimed that life can arise spontaneously. L. Pasteur was sure that these processes could be explained by his microbial theory. However, it had to be proved experimentally and he proved it with an extremely simple but very convincing experiment. He showed that beef broth sterilized by boiling in a flask with a «swan neck», which prevents the ingress of dust and germs, can be kept sterile for a long time. If the neck was broken, the sterilized broth became cloudy over time, indicating microbial contamination.

Biographers claim that L. Pasteur had a firm character and strongly and persistently defended his theory. This allowed him to unite like-minded people who supported his candidacy

in the elections in 1862, when he was elected to the Academy of Sciences. In 1864, L. Pasteur presented the results of several-year research at a conference the Sorbonne and convincingly spoke against spontaneous germination. This solved the philosophical problem of the origin of life and created the basis of the science of bacteriology, which relies on methods of sterilization and aseptic manipulation.

In 1863, L. Pasteur was appointed professor of geology, physics and chemistry at the École des Beaux-Arts in Paris, and five years later he became a professor of chemistry at the Sorbonne. By the early 1870s L. Pasteur had already gained considerable fame and respect in France, and in 1873 he was elected to the French Academy of Medicine.

**Research of infectious diseases and discoveries in the field of immunology.** In 1865, L. Pasteur went to Ales to conduct research on pebrin, a disease that affects silkworms. Because of this disease, the entire French silk industry was under threat. After four years of research, L. Pasteur determined ways to identify the disease and developed recommendations on how to fight it: it was necessary to carefully select healthy undamaged eggs to put an end to the epidemic. The study of silkworms drew Pasteur's attention to the problem of infectious diseases, and this branch of microbiology in the following years completely captivated him.

L. Pasteur made his first important discovery in 1879, studying chicken cholera (today this disease is called avian pasteurellosis, the causative agent of which is *Pasteurella multocida*). He established that during long reseeded, cultures of the chicken cholera pathogen lost their virulence but retained «weakened» pathogenic characteristics for many generations. In collaboration with Emile Roux, he inoculated chickens with the weakened form and demonstrated that the chickens were then resistant to the virulent strain. Then, L. Pasteur directed his experimental work to the problem of immunization and

applied this principle to many other infectious diseases.

In the same 1879, L. Pasteur began to study anthrax, an epidemic of which in France and some other European countries killed a large number of sheep and also affected humans. At that time, German physician Robert Koch announced the isolation of anthrax bacillus. R. Koch and L. Pasteur independently provided definitive experimental evidence that the anthrax bacilli were indeed responsible for the infection.

L. Pasteur applied the principle of vaccination to anthrax. He prepared weakened (attenuated) cultures of anthrax bacilli. In the spring of 1881 he received financial support from farmers to conduct an experiment on immunization of sheep. Vaccination included two inoculations with an interval of 12 days with vaccines of low and higher virulence. Two weeks after vaccination, vaccinated and control (unvaccinated) sheep were inoculated with a virulent strain of anthrax. All animals in the control group died, and all vaccinated animals survived. This convinced many people that Pasteur's vaccine is valid.

After the success with vaccination against anthrax, L. Pasteur began to study rabies. He assumed that the causative agent of rabies was a microbe, but so small that it could not be seen under a microscope. New approaches were needed. L. Pasteur transmitted the infectious agent to rabbits - from animal to animal by intracerebral inoculation. To weaken the invisible agent, he dried the spinal cords of infected animals until the drug became almost non-virulent. Later he realized that instead of creating a weakened form of the agent, his technique actually killed the pathogen. However, the resulting vaccine was effective.

Thus, he created a neutralized agent instead of weakened live microorganisms and paved the way for the development of another vaccines' class known as inactivated vaccines. L. Pasteur, together with his collaborators Albert Calmette, Emile Roux, and Charles Chamberland, tested

the vaccine on dogs and got encouraging results, but did not dare to vaccinate people. An incident forced L. Pasteur to use his vaccine in 1885, when he vaccinated a nine-year-old boy, Joseph Meister, who was bitten by a rabid dog. The vaccine saved the boy's life. This successful medical experiment received great publicity. Subsequently, Pasteur's vaccine saved hundreds of other bite victims around the world, and the era of preventive medicine began.

The solemn celebration of Pasteur's 70<sup>th</sup> anniversary was held at the Sorbonne. Prominent scientists noted his decisive contribution to the development of world science, in particular to microbiology and medicine. The glory of Pasteur's works, recognition of his services to humanity became the impetus for an international campaign to raise funds for the construction of the Pasteur Institute in Paris, the magnificent opening of which took place on November 14, 1888. L. Pasteur was permanent director of the Institute until his last breath. L. Pasteur died on September 28, 1895 with the confidence that his

theories had won and would be useful to mankind for many years. He was buried in the Cathedral of Notre Dame de Paris, but in 1896 his remains were transferred to the Neo-Byzantine crypt in the Pasteur Institute.

The Pasteur Institute became a leading microbiological research center, where like-minded people and students of L. Pasteur worked including world-known microbiologists Emile Duclaux, Charles Chamberland, and Emile Roux. Famous Ukrainian microbiologists Ilya Mechnikov and Sergey Vinogradsky worked at this institute as well.

The importance of Pasteur's works is difficult to overestimate. At first glance, it seemed that his research was aimed at solving practical problems, but each his work was based on deep theoretical concepts and had a philosophical sense. The well-known statement of L. Pasteur: »Thus, once he established the theoretical basis of a given process, he investigated ways to further develop industrial applications« should be a guideline for every scientist in his work.