What Is Life?

The Passionate Dedication of Louis Pasteur

by Denise Bouchard Ham and Roger Ham

Part I of II

A man of genius was needed to bring light in all this darkness. He was to be Pasteur. This man had the rare gift of insight.

You will grant, You will grant, ladies and gentlemen, that there are two ways for the human mind to gain knowledge—reason and imagination. In the modern world, dominated by technology, we are so accustomed to rational progress that we have come to hold imagination in too small esteem. And yet without it there could never be great inventors, any more than there could be great writers and great artists.

Imagination, in the scientific genius, assumes the special form of insight. This is the sudden intuition of a truth without the interposition of reasoning. Insight is what makes the scientists of genius foresee the end to be achieved. . .

What contradictory qualities he must possess! Besides the gift of observation, he must be endowed with imagination, so he must be a poet. . . he must not be narrowly specialized, his knowledge must range over widely varied fields. He must discipline himself to assiduous labor. . . He must confine himself within the bounds of rigorous experiment, requiring him to bridle his imagination. . .[1]

—Pasteur Vallery-Radot, Pasteur's grandson, at the Fermentation Centennial,1957

Blessed is he who carries within himself a God, an ideal, and who obeys it: ideal of art, ideal of science, ideal of the gospel virtues. Therein lie the springs of great thoughts and great actions: they all reflect light from the Infinite.

—Louis Pasteur, 1882[2]

1. As quoted in *The Pasteur Fermentation Centennial (1857-1957)*, by Charles Pfizer & Co., Inc., 1958, pp. 5–6.

2. As quoted by William Osler in the introduction to *The Life of Pasteur*

Mankind owes an inestimable debt to Louis Pasteur (1822–1895), who was trained as a chemist, but who asked, and, in part, answered the question: "What is Life, and what separates it from non-life?" He boldly challenged the entire scientific world in biology, and, later, medicine. Through his passionate, moral commitment to easing the burdens of mankind, he revealed the principles governing the unseen world of microbes, realizing the relatively dormant promise of the invention of the microscope two centuries earlier, and laying foundations for the science of public health upon which we depend.

Pasteur was a Platonist, who inspired those around him to delve into the "unseen" reality of the universe without being bound to any axiom. He knew that the universe is lawful and knowable to the creative mind, and that the creative discoveries of which mind is capable, in turn become a force to change man's relationship to nature.

His life's work directly contributed to an increase in the potential population density of the human species, meaning that through improvements in science and technology, mankind can realize a higher standard of living, a longer, more productive life, and an overall increase in the population of the planet as a whole.

Pasteur never limited himself to a particular field of investigation; he considered himself first and foremost a scientist. He strove to understand the mechanism and life cycle of different diseases, not as a formal interest of study, but with a passionate commitment to saving mankind from them.

His ability to cross the boundaries of crystallography, chemistry, and biology in order to solve a problem would be key to his extraordinary discoveries, but it also brought him into conflict with the scientific establishment that had created those divisions of knowledge. The true history of ideas is the repeated revolutionary change in our fundamental understanding of the universe, but it is too often the case that the professional degrees and reputation of the scientists of one generation rest upon knowledge that has become like an axiom, unchanging and unchallengeable. New knowledge that fits within that structure is acceptable, but that which overthrows those axioms is viewed as a threat and is often violently suppressed. Pasteur's unflinching courage in bringing to life new ideas, and his rigor in proving their efficacy, held greater power than the enemies he made during his lifetime.

> Great spirits have always found violent opposition from mediocre minds.
>
> — *Albert Einstein, letter to Morris Raphael Cohen, March 19, 1940.*

by Pasteur's son-in-law René Vallery-Radot, 1907, p. xvi.

Pasteur's Origins

In the early 1800s chemistry was just emerging as a true science, freed from the pseudoscience of alchemy. New elements were being identified (their number jumping from 55 to 81 during Pasteur's lifetime), and great advances were being made in explaining the chemical processes that occurred in living organisms. Key figures from the Ecole Polytechnique, France's premier scientific school, were studying magnetism, the wave nature of light, constructive geometry, and astronomy. François Arago, Jean-Baptiste Biot, Alexander von Humboldt, Joseph Louis Guy-Lussac, Augustin-Jean Fresnel, Etienne-Louis Malus, Eilhard Mitscherlich, André-Marie Ampère, Gaspard Monge, among other leading scientists, were actively collaborating on these topics around the time Pasteur was born.

Louis Pasteur, the son of a tanner, and great-grandson of a slave who had bought his freedom in 1763, was born on December 27, 1822. Growing up, he gained from his father a love of science, and his parents spent considerable effort and money to educate him. His father hoped that Louis would become a celebrated professor of mathematics or science. But Louis found mathematics dry and formal; his love was science, especially chemistry. As a youth, he also loved art and used pastels to paint his parents, and other citizens of the town.[3]

An outstanding student, Louis studied for a time at several colleges, purchasing along the way a chemistry book by Benjamin Franklin, likely a French translation of *Memoirs of Physics*, published in Paris in 1773. In 1839, he arrived in Paris to study at the Ecole Normale Supérieur, where he excelled in chemistry, physics, and teaching. While there, he became a pupil of Jérôme Balard, who had earned a name for himself when, in 1826, at the age of 24, he discovered the element bromine. This had led to Balard's invitation to teach and experiment at the Ecole. A dedicated teacher and researcher,[4] he insisted that his students invent and create their own scientific apparatus. Balard instantly recognized Pasteur's intuitive genius and had him work as an assistant in Chemistry. A short while later, Auguste Laurent, the Professor of Chemistry at the University of Bordeaux and a corresponding member of the Academy of Science, arrived in Paris to pursue his experiments in crystallography. Laurent likewise took a particular interest in Pasteur, whom he asked to work with him.[5]

3. In November 1863, Pasteur accepted a newly created chair at the School of Fine Arts. He took his students on frequent trips to the Louvre to study the Renaissance masters.

4. Balard slept on a cot in his laboratory so that he would lose no time in his studies.

5. Laurent left the Ecole when he was asked to become the assistant

The Chirality of Crystals and of Life Processes

Developments in crystallography and new techniques in the study of light would enable Pasteur to make his first major contribution to science. In 1846, he decided to make chemistry his life's work: "When I began to pursue specific research, I sought to strengthen my abilities by studying crystals, anticipating that this would provide me with knowledge I could use in the study of chemistry."[6]

Molecules constitute the building blocks of all matter; studying their organization can help reveal their specific function. Since atoms and molecules are too small to be seen, crystals were studied extensively as a way of gaining insight into the spatial arrangement of their atoms and the changes that occur through chemical reactions. For 19th-century scientists, crystallography was a way to reveal the unseen chemical bonding of molecules through the geometrical form of the crystals. In 1819, German chemist Eilhard Mitscherlich developed his theory of *isomorphism* (meaning "having the same shape"), which grouped elements based on the similarity of the compounds and crystals they formed.

Related to this was another tool for seeing the invisible, which became central to Pasteur's discoveries: polarization of light, which limits the passage of light waves through a polarizing medium according to its orientation. The waves in rays of sunlight normally vibrate in all directions, or planes, perpendicular to the motion of the rays. However, in 1808, it had been shown that when light is reflected off water or another flat surface, the waves in the resulting glare all vibrate in one plane; the light is said to be polarized.

A paradox in the phenomenon of polarization led Pasteur to his first major discovery and to subsequent breakthroughs in the science of life. In 1811, François Arago had discovered that some crystals, such as quartz, could rotate the plane of polarized light either to the right or the left, clockwise or counterclockwise with respect to the motion of the light. This was followed in 1815, when Jean-Baptiste Biot—a pioneer in the study and use of polarized light—observed that certain liquids, including turpentine and sugar solutions, could also rotate polarized light. Such substances were called "optically active." A device known as a polarimeter was developed to measure the degree of rotation of light, as it passed through an experimental solution.

lecturer to Jean-Baptiste Dumas at the Sorbonne. At that time, Dumas was the most celebrated chemist in France, a member of the Academy of Sciences and the founder of the Central Institute for training French engineers.

6. Patrice Debré, *Louis Pasteur*, p. 33.

Leatherhead Quartz Crystals

Large quartz crystals. Quartz, an asymmetrical crystal, rotates the plane of polarization of light passing through it.

Life and non-life

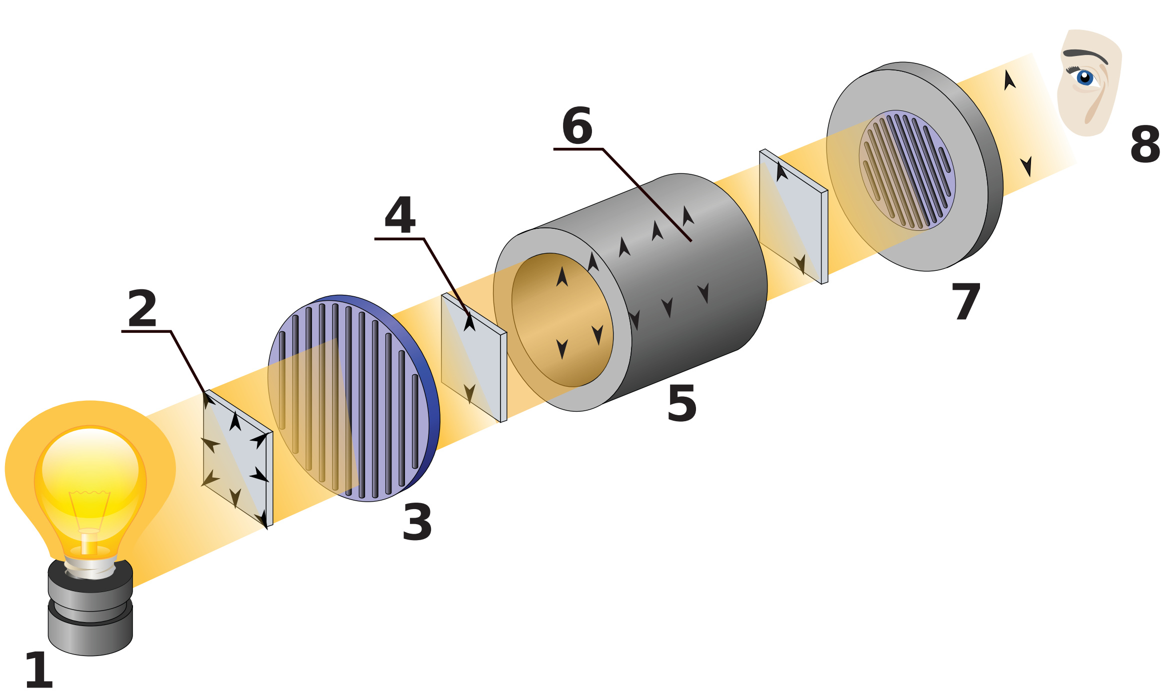
For centuries, crystallized salts called tartrates, formed from tartaric acid in grapes, had been a familiar sight in wine vats, and occasionally on the cork in a bottle of wine. In 1819, a second, very rare form of tartaric acid crystals was found in a few wine vats. These slender, needle-like crystals were called paratartaric or racemic acid. Both kinds of crystals had exactly the same chemical composition and properties, indicating that the arrangement of the atoms should be identical.

In 1832 Jean-Baptiste Biot observed that a solution of tartaric acid rotated the polarization of light to the right, but it was not known why. And twelve years later, Eilhard Mitscherlich submitted a startling report to the French Academy of Sciences on tartaric and paratartaric acid: these acids, although seemingly identical, had different effects on polarized light.

Liquid solutions made from tartaric acid crystals rotated light to the right, as did the crystals themselves, but solutions made from paratartaric acid crystals did nothing! This paradox sparked a tremendous debate in the chemical community. If every physical test known to science indicated that the two compounds were identical in every way, what could cause this optical difference?

Pasteur took up the challenge.

Drawing upon his extensive study of crystals, his insight was to treat these chemical crystals, formed in the course of fermentation of grape juice, as if they were naturally occurring mineral crystals, like quartz. Symmetrical crystals do not rotate polarized light, but dissymmetrical crystals like quartz *do* rotate polarized light. Just like your right and left hands, some crystals can form mirror images of one another. This property of "handedness" is called *chirality* (from the Greek word for *hand*). Pasteur was the first scientist to show that when examined closely, tartrate crystals revealed small secondary

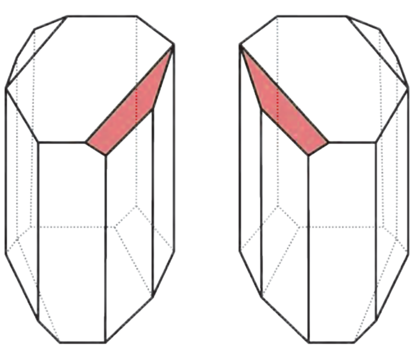


CC/by-sa-3.0 Wikimedia user Kaidor (including elements from user Jacob Hnri 6)

Schematic of the functioning of a polarimeter. Light first passes through polarizing filter **3***, after which all of its oscillating waves are in the same (vertical) plane. As it passes through the test sample* **6***, the plane of polarization is rotated. The angle of rotation is measured by a rotating polarizing filter* **7***, manipulated by the experimenter.*

facets on one side only, making them dissymmetrical. In the same way that quartz crystals rotated polarized light, tartaric acid rotated the plane of polarized light to the right, even when in a solution, because every molecule was right-handed. He had proven that it was the dissymmetry of the molecule itself which caused the rotation. But a question remained: why didn't paratartrate crystals, if they were chemically identical, rotate light?

Pasteur initially thought that the optical inactivity of the paratartrates must be due to a symmetry in the crystalline structure. To test his hypothesis, he allowed a solution of paratartaric acid to crystallize by drying. Pasteur then painstakingly examined each tiny crystal and discovered that these crystals also had dissymmetrical facets. But this time he found both right- and left-handed versions of the crystal! He sorted them into piles of right- and left-handed crystals and then made solutions from each pile. Much to Pasteur's delight, they each rotated polarized light, one to the right and one to the left. The original paratartrate solution hadn't rotated light, because an equal number of left- and right-handed molecules had been formed, canceling out any rotation.



21st Century Science and Technology

Left- and right-handed versions of tartaric acid.

Pasteur was so excited by his discovery that he ran from the lab and exclaimed to the nearest teacher that he had made a wonderful discovery.

The matter was referred to Jean-Baptiste Biot, by then a respected professor, in his 70s, at the Ecole and a member of the French Academy of Science. Biot was initially skeptical of such a profound claim by a 25-year-old assistant chemist. Pasteur reported:

> He [Biot] summoned me to repeat the decisive experiment before his eyes. He gave me the paratataric acid he had carefully studied himself beforehand and which he found to be perfectly neutral toward polarized light... We left the liquid in one of the slow evaporation cabinets he had in his laboratory, and when it had yielded about 30-40 grams of crystals, he asked me to come to the Collège de France in order to gather them and to separate out the right-handed and the left-handed ones according to their crystallographic character under his eyes. He again asked me if I was really saying that the crystals I would place to his right would rotate to the right and the others to the left. This done, he said he would do the rest. He prepared the carefully weighed solutions in the proper amounts, and

Producing Specific Isomers

The production of either left- or right-handed isomers can be done in a number of ways. Pasteur's original insight led him to separate, by hand, the tiny crystals formed by evaporation of the racemic mixture of paratartrates, a technique today called chiral separation. It is usually easier to start with a chiral building block or add one during the synthesis process. If the desired product is not too dissimilar, synthesis can begin with a sugar or amino acid molecule which already has the desired chirality. Or a chiral subunit can be added during the manufacturing process to produce a product with only that chirality. In the case of the cholesterol-lowering medicine Lipitor, this chiral auxiliary is removed at the end of the process, having done its job. A chiral catalyst or enzyme (usually biological in origin) can be used to selectively synthesize a higher proportion of the desired enantiomer. After Pasteur combined a racemic mixture of ammonium tartrate with a *Penicillium glaucum* mold, he found that only the left-handed tartrate remained. This was the first known use of what is now called kinetic resolution of enantiomers. One final technique converts an equal mixture of enantiomers into an equal mixture of diastereomers (non-mirror image molecules which still contain the identical atoms). The non-mirror image molecules then have different chemical properties which allow them to be separated using differences in their boiling points, solubility, etc.

In the case of Thalidomide, a drug used to treat morning sickness, none of these measures would have prevented the birth defects, because Thalidomide can interconvert *in vivo,* switching from one enantiomer to the other. Today, Thalidomide is used to treat leprosy and certain cancers, under strict controls to prevent contact with pregnant women.

Flickr/Luciana Christant

A reminder of the importance of chirality: children with birth defects caused by Thalidomide.

when the time came to look at them in the polarization apparatus, he again called me to his laboratory. He first placed into the apparatus the most interesting solution, namely the one that was supposed to rotate the light to the left. Without even taking a measurement, Biot realized from the mere sight of the two images in the polarimeter, one ordinary and one extraordinary, that there was indeed a strong rotation to the left. Then, the illustrious old man, visibly moved, took me by the arm and said: "My dear boy, I have loved science so much all my life that this stirs my heart."[7]

Pasteur's discovery was published to great acclaim in 1848, just before his 26th birthday. The tartrates were the first molecules ever isolated in right- and left-handed forms. While the components of living and non-living matter could be chemically identical, Pasteur's further research revealed that virtually every active and naturally occurring biological molecule was exclusively either right- or left-handed. This "asymmetrical force," as Pasteur called it, operates only in living organisms and is the most dramatic boundary condition separating the chemistry of non-living from living matter.

Laboratory synthesis of any dissymmetrical molecule produces equal amounts of each mirror form (*isomer* or *enantiomer*), forming solutions that are therefore optically inactive. *Living* processes, however, uniquely produce only one of the possible forms. Over the next five years, he continued to study isomerism, in the process giving birth to stereochemistry, which studies the three-dimensional shape of molecules.

When both enantiomers exist in living processes, they have different roles. As a modern example, one form of the sugar substitute aspartame is 200 times sweeter than sucrose, while the identical mirror image molecule is bitter.

The infamous drug Thalidomide was prescribed from 1957 to 1961 to relieve nausea suffered by pregnant women, but was banned after severe birth defects were linked to the drug. Later research showed that the right-handed version did relieve nausea, while the left-handed version was responsible for the birth defects.[8]

Today a multi-billion dollar industry is dedicated to increasing the proportion of the desired enantiomer produced through the complex series of chemical reactions required to mass-produce these complex molecules. (See box: Producing Specific Isomers)

The Secret of Fermentation

These discoveries paved the way for Pasteur's entry into research in biology, beginning with an incident in

7. Debré, p. 48.

8. At least one-third of all drugs produced today are chiral, including Ibuprofen, Naproxin, Lipitor, Zocor, Paxil, Zoloft and Nexium. In the case of Ibuprofen, only one enantiomer is biologically active, so it can be sold as a racemic mixture of both forms, but this is not possible with Naproxin, in which the left-handed form is a pain reliever, but the right-handed form is a liver toxin which must be excluded during the manufacturing process.

Fermentation

Thousands of years ago, the Greeks and Egyptians made wine and beer; other ancient cultures made rising bread. The knowledge of how all this occurred, however, wasn't discovered until the 19th century. The view taken by the ancients, as well as Pasteur's contemporaries, was that this process was simply a chemical action. Even in Pasteur's time, he was attacked by the prominent German chemist Justus von Leibig, who believed it was simply the action of oxygen, and others who refused to consider this as a life-process.

Great advances were being made in explaining the chemical processes that occurred in living organisms. The great French chemist, Antoine Lavoisier, had shown that the chemical "combustion" in living animals is quantitatively identical to that occurring in a furnace: a carbon-based fuel combines with oxygen, producing energy and carbon dioxide. He also showed that sugar, the raw material for fermentation, could be broken down into alcohol, carbon dioxide, and water by simply dropping droplets of a sugar solution on heated platinum.

1856. At that time, he lived with his wife and two children in Lille, a key industrial center, where he had accepted, at age 31, the chairmanship of the science department and position as Dean of the University.

It was common for working men and industrialists alike to sit in and listen to Pasteur's classes, especially the weekly lecture he gave on chemistry and its application to industry, which were always immediately followed by a visit to a local factory. Pasteur always insisted upon testing, on a large scale, what he had witnessed in the laboratory. This close connection between his laboratory and industry cohered with his immediate sharing of each of his discoveries.

Thus it transpired, that the father of one of Pasteur's students, a leading producer of alcohol from beetroot juice, sought out the professor's help in finding the cause of failed fermentation, where the juice became acidic and fetid, a problem of considerable economic importance to wine- and beer-makers. Pasteur immediately brought his method and microscope to aid in what was to become his first foray into biology and a crucial part of his life's work. To chemists, it seemed absurd, or at least strange, to attempt to study a chemical reaction with a microscope, but Pasteur was always ready to innovate.

Nineteenth-century fermentation vats.

The beet juice was placed in huge wooden vats, where the natural sugar fermented into alcohol. Upon examination of the juice under his microscope, Pasteur observed round globules that grew and multiplied—yeast. Chemical analysis also showed the appearance of optically active amyl alcohol. Based on his work in crystallography and optical activity, these two observations immediately led Pasteur to the hypothesis that the yeast was itself central to the fermentation process.

This was a breakthrough. Although earlier scientists had observed that yeast—a fungus widely distributed outdoors—was present in fermentation, it was thought to be either a product of fermentation or merely a catalyst in a purely *chemical* process. The suggestion that there was a "vitalistic," life force in fermentation was ridiculed by the scientific establishment, and even viewed as a dangerous step backward in science. But Pasteur, not allowing preconceptions to influence his work, recognized that this was no simple chemical reaction; the *living* yeast was converting the sugar into alcohol, carbon dioxide, and water, in order to release energy to fuel its own cellular activity and reproduction.

Pasteur also observed a slimy coating on the surface of the juice in some vats, accompanied by a sour smell. Upon microscopic examination, he saw not the round yeast he expected, but instead, huge numbers of tiny, black rods. Pasteur concluded that this, too, was a life process and that the rod-shaped organisms were a new class of yeast, which, he found, produced lactic acid instead of alcohol, ruining the entire vat of juice. Through months of study, he was able to show that some yeast was responsible for the fermentation of sugar into alcohol in wine and beer, while other yeasts or bacteria were responsible for converting alcohol to acetic acid in vinegar, and also lactose to lactic acid in yogurt. It was these unintended microbes that caused ferments to sour, not simple chemical reactions.[9] While he now understood

9. Pasteur referred to fermentation as "life without oxygen." In the development of life on Earth, this was the mode of respiration and energy production in organisms prior to the emergence of photosynthe-

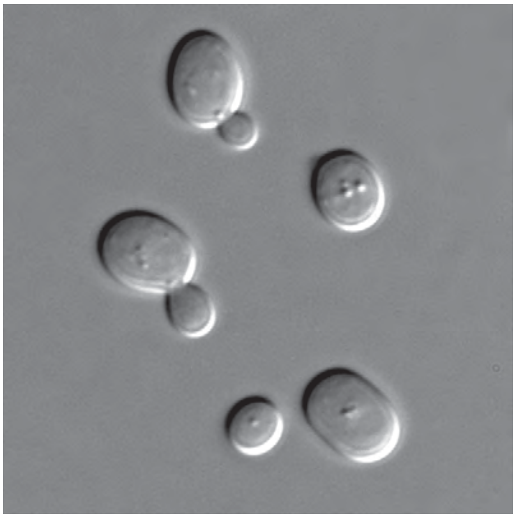
why the presence of unwanted microorganisms would ruin the fermentation, his solution took a few more years to develop.

In 1857, Pasteur published a paper on lactic fermentation which laid out all the essential concepts of his discovery, a paper which has been referred to as the birth certificate of microbiology, due to his key insight that fermentation is *caused* by living organisms. His paper concludes:

> My present and most fixed opinion regarding the nature of alcoholic fermentation is this: 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 there being simultaneously the organization, development, and multiplication of the globules, or the pursued, continued life of globules which are already formed.[10]

In order to kill most of the unintended bacteria present, without damaging taste or nutritional value, in 1862 Pasteur conducted his first experiments to test the effect of briefly heating wine and beer. This dramatically increased the "shelf life" of these products. By 1865, he had developed what we now know as the process of *pasteurization*. The 1876 publication of Pasteur's "Studies on Fermentation: The Diseases of Beer, Their Causes, and the Means of Preventing Them" was a huge leap forward in the scientific understanding of beer-making, followed in subsequent years by the pasteurization of milk and many other products. The book was translated and published in English in 1879, and was studied by brewers around the world. In Copenhagen are found Pasteur Street and a statue of the great scientist, thanks to whom the Carlsberg Brewing Co. successfully sent a shipment of beer all the way to India.

A van Leeuwenhoek microscope, circa 1668



Budding yeast cells, the cause *of fermentation.*

The process of pasteurization is probably the only universally known discovery by Pasteur in the world today, a sorry "sign of the times" in which we live.

Germ Theory

Men have speculated since ancient times that living agents could enter the body and cause disease, but until the invention of the microscope in the 1660s, that speculation could not be verified. However, as we will see, it was not the power of the microscope to enhance vision, but the power of insight, the rigor of method, and the courage to challenge accepted precepts, that led to the breakthroughs in knowledge upon which our health today depends. Prior to the mid-nineteenth century, disease was generally viewed as a miasma, akin to a poisonous gas, which could infect many people, but was not transmitted from person to person—this, although Antonie van Leeuwenhoek of Holland had opened up a new world of perception with his first microscope in 1668. He was the first to see and describe bacteria, yeast globules (which he believed to be nonliving, starchy structures), drops of water teeming with new forms of life, and the circulation of blood corpuscles in capillaries. But who could know what it all implied?

sis and its release of oxygen into the atmosphere, and is also the mode of energy production that occurs during brief strenuous muscular exertion. Human bodies can produce energy without oxygen, forming lactic acid, the cause of both sore muscles, and sour milk.

10. Pasteur, "Mémoire sur la fermentation alcoolique," *Annales de Chimie et de Physique* (1860), **58**:3, 359–360, as translated in Joseph S. Fruton, *Proteins, Enzymes, Genes: The Interplay of Chemistry and Biology* (1999), p. 137.

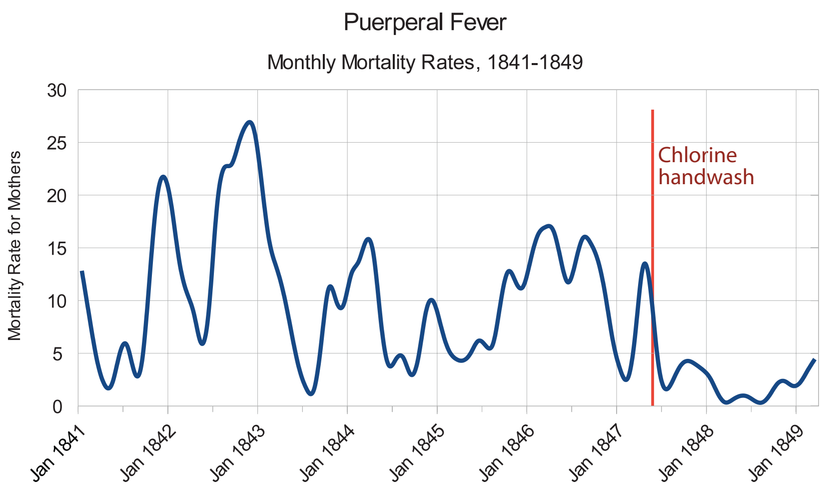
The case of the Italian Agostino Bassi is illustrative. He is often credited with having stated the germ theory of disease for the first time, based on his observations of the lethal and epidemic muscardine disease of silkworms. In 1835 he blamed the deaths specifically on a contagious, living agent, visible to the naked eye as powdery spore masses (later named *Beauveria bassiana* in his honor).

But despite this and other early insights, a scientific understanding of the nature of microbes, how they invaded the body and actually caused disease, remained elusive.

Under these conditions, battlefield and even hospital medicine were atrocious. The majority of wounded soldiers died of infection, not from actual combat. Doctors rarely washed their hands or surgical instruments, and bandages were taken off the dead and immediately reused on wounded soldiers.

Women giving birth faced similar odds. At the Paris Hospital, the death rate among women in labor was 20–25%. In 1847, Ignaz Semmelweis, a Hungarian obstetrician working at Vienna's Allgemeines Krankenhaus (hospital), began seeking a reason for the dramatically high incidence of death from puerperal fever (also called childbed fever, most commonly caused by *Streptococcus* or *Staphylococcus* bacteria) among women who delivered at the hospital with the help of the doctors and medical students. In contrast, births at home, attended by midwives, were relatively safe. Investigating further, Semmelweis observed that the delivery physicians often came directly from autopsies performed on mothers who had died the previous day. Asserting that puerperal fever was a contagious disease and that "cadaverous particles" were implicated in its development, Semmelweis made doctors wash their hands with chlorinated lime water before examining pregnant women. Mortality from childbirth fell to less than 2% at his hospital. Nevertheless, he and his theories were ignored or viciously attacked by most of the Viennese medical establishment. A typical response was, "Doctors are gentlemen, and gentlemen's hands are clean."[11]

11. A 1938 film, based on Semmelweis' work, *That Mothers Might Live*, was awarded the Oscar for Best Short Film.



Mortality rates from Puerperal Fever among women giving birth at the Vienna General Hospital. Note the plunge in deaths after Semmelweis instituted simple hand washing with chlorinated water in 1847.

Spontaneous Generation

In 1858, at the same time Pasteur was doing his ground-breaking work on fermentation, he became embroiled in a bitter fight over the nature and origin of life itself. Adherents of spontaneous generation, led in France by Félix-Archimède Pouchet, the director of the Natural History Museum of Rouen, believed that life could arise spontaneously from non-life. It was not a new debate. Aristotle had asserted that life could arise spontaneously out of dirt and dust: "every dry body which becomes moist and every humid body which dries up breeds life."[12] In the early 1600s, Flemish physician and alchemist Jan van Helmont wrote: "the emanations rising from the bottom of marshes bring forth frogs, snails, leeches, herbs, and a good many other things." He also maintained that mice could arise from corn and a dirty shirt left in a vessel for three weeks.[13]

Pasteur knew he was entering a hostile arena. His colleague and good friend Jean-Baptiste Biot begged him not to enter the fray. It is far more difficult, he argued, to prove something *cannot* exist than to prove something *does* exist. But Pasteur knew, from his work on crystallography and fermentation, that this fundamental issue would generate valuable insights far beyond questions of frogs and mice. His entrance into the scientific battle increased its prominence, and all of France began to follow the experiments made by each side. In April, 1860, the *Moniteur Scientifique* asked: "What will be the outcome of this battle of the giants?"[14]

Pasteur's grandson, Pasteur Vallery-Radot, later wrote of the contest:

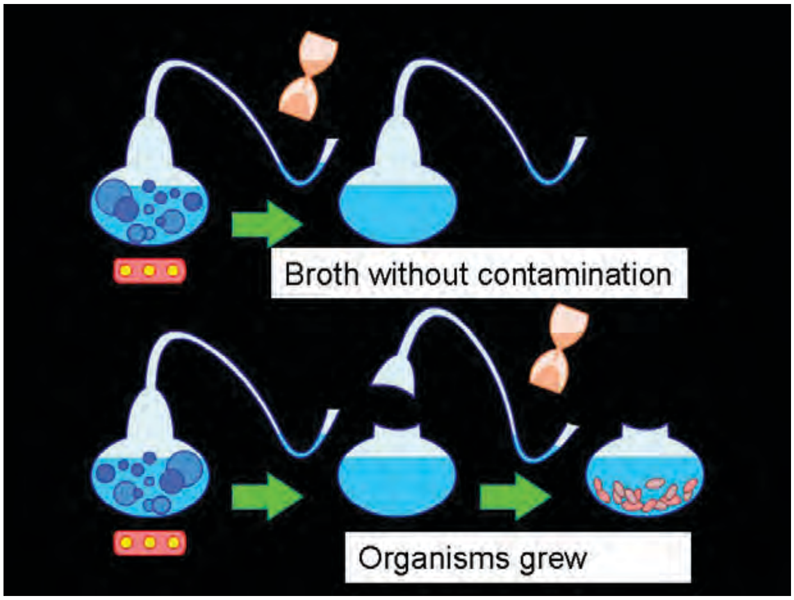
> While Pasteur had no preconceived idea and simply expected from the experiment the answer to a given problem, Pouchet wanted the experiments to confirm

12. As quoted in Pasteur Vallery-Radot, p. 58.

13. Pasteur Vallery-Radot, pp. 58–59.

14. Debré, p. 163.

what he already believed "by meditation." Thus Pouchet violated the basic rule of a scientific experiment, which is that the gravest error lies in the desire to confirm what one believes; indeed one must always experiment without prejudging the outcome. As Bossuet said: "It is the worst aberration of the mind to believe things because one wishes them to be so." . . . What polemics and controversies to establish definitely the doctrine of the non-spontaneity of germs! Pasteur devised the most ingenious experiments, revealing the remarkable fertility of his imagination, his prowess as an experimenter, and at the same time displaying his forceful argumentation. . . challenged the views of his peers, overwhelmed his opponents with experiments. . . He smashed their objections one after another.[15]



Representation of how Pasteur's swan-necked flask experiments disproved spontaneous generation.

Pouchet believed that germs were very rare and could not account for all the organisms seen. He argued that if germs were everywhere, the air would be so thick that it would have the density of iron.

Pasteur wrote to Pouchet that the results he had attained were:

> . . . not founded on facts of a faultless exactitude. I think you are wrong, not in believing in spontaneous generation (for it is difficult in such a case not to have a preconceived idea), but in affirming its existence. In experimental science it is always a mistake not to doubt when facts do not compel affirmation. . . In my opinion, the question is wholly untouched by decisive proofs. What is there in air which provokes organization? Are they germs? Is it a solid? Is it a gas? Is it a fluid? Is it a principle such as ozone? All this is unknown and invites experiment.[16]

Pasteur, as always, took a rigorous experimental approach, using the skills learned from Prof. Balard in making his own instruments, with an ingenious invention to prove his germ theory. He created a new kind of flask. It looked like a bulb with a doubly curved, thin opening resembling the neck of a swan. In it he put water, sugar, and yeast. He heated the flask until it boiled and then simmered the mixture in order to kill any organisms present.[17] After allowing the flask to cool, he inserted a small wad of cotton into the end of the neck. The long, narrow neck allowed air to enter, while preventing any germs or dust from entering the flask. The liquid inside the flask remained clear and free of organisms for months or years. When he broke the neck, or tilted the flask allowing some of the solution to run down the neck and back into the flask, microbes were allowed to enter the flask, multiply and make the solution cloudy.

Pasteur concluded that germs in the air had to be introduced to the flask to produce life. To further refine his hypothesis, he took his experiments 6500 feet in elevation up Mont Blanc, where the air was purer than that in the city. When the sealed, sterile flasks were opened high on the mountain, fewer of the flasks became cloudy. This confirmed for Pasteur that air in some areas was nearly germ-free and that germs were the sole source of life in the experiment. He repeatedly demonstrated that a fermentable liquid, if sterilized and exposed to only the purest air, would lie dormant.

Pouchet made new challenges and experiments, similar to Pasteur's, but, without the latter's rigorous controls, always resulting in solutions teeming with germs. Life could start in any place, he asserted, and growth is found in every case, regardless of the quality of the air used.

When the Academy of Science was called to test both Pasteur's and Pouchet's experiments, Pouchet gave up in the middle of his experimentation, while Pasteur had produced over 60 successful flasks. Still, the debate continued, and on April 7, 1864, Pasteur gave a lecture at the Sorbonne in Paris. Referring to his swan-neck flask experiments, Pasteur said, "Never will the doctrine of spontaneous generation recover from the mortal blow

15. Pasteur Vallery-Radot, p. 62.

16. René Vallery-Radot, p. 94.

17. The Italian scientist Lazzaro Spallanzani had shown in the 18th century that boiling killed these tiny creatures.

struck by this simple experiment." He went on to say:

> As I show you this liquid, I too could tell you, "I took my drop of water from the immensity of creation, and I took it filled with that fecund jelly . . . full of the elements needed for the development of lower creatures. And then I waited, and I observed, and I asked questions of it, and I asked it to repeat the original act of creation for me; what a sight that would be! But it is silent! It has been silent for several years, ever since I began these experiments. Yes! And it is because I have kept away from it, and am keeping away from it to this moment, the only thing that it has not been given to man to produce, I have kept away from it the germs that are floating in the air, I have kept away from it life, for life is the germ, and the germ is life."[18]

Pasteur received a standing ovation from the large majority of attendees. His experiments regarding germ theory were not the first, but they were the most rigorous. The sterilization techniques Pasteur developed led to the autoclaving of instruments (using steam at high pressure to sterilize), invented by one of Pasteur's students, Charles Chamberland, which drastically reduced infection caused by surgical instruments.

Despite these and other results, the theory of Spontaneous Generation would still have supporters for some decades. In 1882 (20 years later!), Louis again attacked the remaining supporters of Spontaneous Generation and the religious leaders who supported their claim: "This has nothing to do with religion, or with philosophy, or with systems of any kind. Assertions and *a priori* views do not count; we are dealing with facts." Looking back at the end of his life, Pasteur said:

> Spontaneous Generation is something I have been looking for without finding it for twenty years. No, I do not consider it impossible. But on what grounds do you think you can say that it was the origin of life? … Who tells you that the steady advancement of science will not oblige scientists living a hundred years, a thousand, ten thousand years from now... to maintain that life has existed for all eternity, but not matter? You move from matter to life because your current intelligence, so limited in comparison with the intelligence of future naturalists, tells you that it cannot think otherwise. Who can assure me that in ten thousand years it will not be considered impossible to think that life does not change into matter?[19]

Flickr/guojerry

A silkworm and cocoon, spun from a single strand of silk, one kilometer long.

Rescuing the Silk Industry

Pasteur's success in revealing the cause of diseases of wine, milk, vinegar, and beer, had led him to conclude that such "microbes" were also responsible for the diseases afflicting animals and man—a revolutionary idea. As in the beet root case, he found himself called upon to solve an important agro-industrial problem.

The French had been involved in sericulture—the rearing of silkworms—for several centuries. By the middle of the nineteenth century, annual production had reached 26 million kilograms of silk. But disaster struck. An epidemic disease ravaged the silkworms, collapsing French production to just four million kilograms by 1865. At first, silkworm rearers had resorted to buying eggs abroad, but the disease had spread globally, and only the island nation of Japan seemed to have avoided the scourge. Even healthy imported broods succumbed to the disease within a few years of their arrival in France. The government received a petition signed by thousands of French mayors, councilmen and landowners, demanding that the government send an entomologist or veterinarian to find a cure. Pasteur's former teacher, Jean-Baptiste Dumas, a member of the French Senate as well as a scientist, believed that Pasteur's fermentation experience uniquely qualified him. He begged Pasteur to take the job, despite the fact that he was a chemist and had never even seen a silkworm! To Pasteur's protests, Dumas replied: "All the better, for you will have no preconceived ideas and will be guided by the results of your own work."[20] Pasteur was to spend much of the next six years working on this problem.

The disease killing the silkworms was called *pébrine* (after the French word for pepper), because black spots

18. Debré, p. 169.

19. Debré, pp. 175–176.

20. Fishbein, p. 30.

appeared on the worms. Also, their tissue contained minute, oval, shiny corpuscles 2–3 micrometers in length. If these corpuscles were found in a sampling of eggs, the entire brood was likely to fail. Outbreaks could occur at any stage in the silkworms' development, often among apparently healthy worms. One batch of eggs could produce healthy worms, while a second batch of eggs, kept under identical conditions and fed the same mulberry leaves, could produce solely diseased worms.[21]

Pasteur's visits to many silkworm rearers revealed a vast number of theories with an equal number of "experts" to explain them. "Cures" included applying chlorine gas, sulphur, coal dust, wine, rum, acids, tar vapors, numerous "secret" ingredients, and even electrical currents. Yet the destruction continued. And even in the major sericulture center of Alais, no one had either seen, or had even expressed the desire to see, under a microscope, any of the corpuscles whose existence had been known since 1849.

"I decided," wrote Pasteur, "to adopt a line of approach very different from that of my predecessors. I would concentrate my attention on one given point, the most significant I could find, and not give up my study of it until I had established a certain number of principles which would allow me to advance with safety into the labyrinth of preconceived ideas. . . I will, for the moment, direct my attention exclusively to an examination of the questions raised by the presence of the corpuscles."[22]


Joseph Lister, the English physician who championed antiseptic medicine.

Thus in 1865, Pasteur began a series of controlled experiments to develop a clear chain of causation. He microscopically examined the tissue of eggs, worms, pupae, and moths at all stages of life and correlated these findings with the future health of the individual worms and the quality of the silk produced from their cocoons. This was no easy task, because nearly all of the moths and pupae were infected. In February 1866, he brought two former students to Alais, whom he trusted to be his assistants, Désiré Gernez and Eugène Maillot, later joined by Emile Duclaux. Pasteur was in the process of creating a science youth movement from among the young doctors and scientists not tied to the old assumptions and doctrines held as sacrosanct by the high priests of French medicine.

Pasteur and his assistants rose at 4:30 every morning. The first order of business besides checking on the silkworms, was to painstakingly sanitize the work area completely. Everything and every surface, including the walls, had to be hygienic to rule out contamination by dirt and microbial dust.

Pasteur was able to prove that the disease was contagious and transmitted by a parasite, and then worked to show how the disease was transmitted. He developed a method of testing about 100 pupae and 100 moths that allowed him to predict the health of 25–30,000 eggs, and by 1867, his methods of testing and sanitation were applied with excellent results, showing that the environment played a huge role in the spread of the disease.

But, other silkworms died that were free of the corpuscles. This paradoxical situation gradually led him to conclude that there was a second disease called *flacherie*, in which the worms became soft and flabby. Pasteur studied this disease from 1867–69 and found organisms in the worms' intestines which resembled the fermentation agents he had already studied. The bacteria could be transmitted via the mulberry leaves fed to the worms, especially if the leaves were cut, wet, or had excrement from the worms.

Pasteur biographer Patrice Debré describes the disease and its cure: "It should be pointed out that the description of the multiple causes that facilitate the proliferation of the microbes responsible for flacherie, whether they be bacteria or viruses, was less important than the fact that Pasteur had established that this was indeed an infection and that he had attempted to prevent it. On the basis of his finding, he proposed a series of hygienic measures, including better ventilation for the nurseries, scrubbing the floors, careful management of the silkworms' food, the picking and conservation of the mulberry leaves, and prevention of heat and humidity from pervading the atmosphere of the breeding chambers."[23]

---

21. The silkworm goes through two metamorphoses within the cocoon, forming a chrysalis—a kind of mummy—and later a pupa, which finally emerges as a moth to continue the cycle by laying a new generation of eggs.

22. Nicolle, p. 114.

23. Debré, p. 205. Debré writes: "Emil Roux later wrote of Pasteur's book on the silkworm that it was a veritable guide for anyone who undertook to study contagious diseases. Pasteur was aware of this and pointed it out to the physicians. He never failed to say to those who came to work in his laboratory, chosen by him to collaborate in his study of infection in animals: 'Read the Etudes sur la maladie des vers

The potential value of this anti-septic approach was quickly recognized by the English physician Joseph Lister, who became a strong supporter of Pasteur and began corresponding with him in 1874. Practicing in Scotland, he began a campaign for a germ-free surgical environment. Lister ended the practice of re-using bandages, demanding absolutely clean linen, and successfully used carbolic acid to sterilize wounds and the entire operating theater. Like Semmelweis earlier, Lister became a target of attack by the medical establishment. Yet, his success rate was more than double that typical beforehand. A turning point in the acceptance of Lister's methods came in 1876, when he was invited to speak at the International Medical Congress held in conjunction with the U.S. Centennial Celebration in Philadelphia. Among those in the audience was Robert Wood Johnson, who was already greatly influenced by Lister's ideas and went on to manufacture and market the first commercial sterile surgical bandages. He and his two brothers founded Johnson & Johnson in 1886. An increasing number of physicians began to adopt Lister's aseptic approach to surgery and wound treatment, dramatically reducing mortality rates.

For the first time, the origin of a disease in a living organism had been traced to the action of a microbe. Pasteur's new hypothesis saved the French silkworm industry and the livelihoods of the farmers who were nearly wiped out by the disease. He considered the potential of using the killing power of certain microbes to eliminate harmful insects or parasites that ravaged crops. Equally important, Pasteur's work on silkworms would help him tackle other biological problems, saving mankind from a host of diseases. His experiments led him to believe that addressing filthy conditions and over-crowding were an essential aspect of treating human disease. Pasteur fought to organize the French government to provide adequate supplies of fresh water as well as a sewage system, to prevent the spread of deadly diseases like cholera. While others had discussed the presence of microbes and their possible role in disease, Pasteur's work was the beginning of a rigorous and powerful germ theory because of his commitment to conquer these diseases. Through meticulous experimentation he developed an arsenal of ideas which fundamentally shifted the battle.

The history of science is incomprehensible without recognition of the role of morality such as Pasteur's. In contrast, the British parson and East India Company employee Thomas Malthus (1766–1834) explicitly rejected the idea that human society was perfectible and saw famine and disease as "natural" checks on the growth of population. He attacked doctors who sought to cure diseases, and instead, encouraged over-crowded and filthy conditions in the slums of London, in order to increase the death rate among the "undesireables." Thus are the scientific and economic policies of nations intimately related to their view of the nature of Man.

à soie, for I think that it will be a good preparation for the work we are about to undertake.'"

In Part II (to appear in the next issue), we will study Pasteur's extraordinary years of discovery, focusing on his triumphs over anthrax and rabies. A short companion article, will look at the impact of Pasteur's work on later scientists as well as investigations of chirality being undertaken today.

Bibliography

Byrd, Hiram, 1910, "An Appreciation of Louis Pasteur," oration before the Florida Medical Association, The Record Co., St. Augustine, FL.

Congrès du centenaire de la découverte du processus des fermentations par Pasteur, *The Pasteur Fermentation Centennial*: 1857–1957, Charles Pfizer & Co., NY, 1958.

Cuny, Hilaire, *Louis Pasteur, The Man and his Theories*, translated by Patrick Evans, Souvenir Press, 1965.

Debré, Patrice, *Louis Pasteur,* originally published by Flammarion in 1994, English translation by Elborg Forster, Johns Hopkins, 1998.

Dubos, René, *Pasteur and Modern Science,* Anchor Books, 1960.

Duclaux, Emile, *Pastuer: The History of a Mind,* W.B. Saunders Co, 1920.

Fishbein, Morris, Louis Pasteur: *The Man and His Work,* 1925, Haldeman-Julius Co, Girard, KS, 1924.

Geison, Gerald L., *The Private Science of Louis Pasteur,* Princeton Univ. Press, 1995.

Hammerman, W. (1986), "Louis Pasteur, Father of Today's Optical Biophysics," *Fusion,* Vol. 8, No. 5.

Metchnikoff, Elie, *The Founders of Modern Medicine: Pasteur, Koch, Lister,* translated by David Berger, Walden Publications, 1939.

Nicolle, Jacques, *Louis Pasteur, The Story of His Major Discoveries,* English translation published by Basic Books, 1961.

Vallery-Radot, Pasteur, *Louis Pasteur, A Great life in Brief,* translated by Alfred Joseph, Alfred A. Knopf, NY, 1958.

Vallery-Radot, René, *The Life of Pasteur,* translated by R. L. Devonshire, The Sun Dial Press, Garden City, NY, 1937.

Zahm, Rev J. A., "Louis Pasteur and His Life-Work," *The Catholic World,* Vol. LVI, No. 334, Jan 1893.

Yassine Mrabet