The Legend of Louis Pasteur

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For a scientist, making a seminal contribution even in one area of science is a matter of joy and pride. To be able to start several fields and contribute significantly to all of them is indeed a rare feat. Louis Pasteur belongs to this rare breed of scientists. He was a chemist, microbiologist, immunologist, and biotechnologist, all rolled into one. He founded the field of stereochemistry, established the foundations of modern microbiology, disproved the theory of spontaneous generation, demonstrated the microbial basis of fermentation, and derived vaccines against several bacterial and viral diseases. His contributions to human welfare in one lifetime are more than what others can aspire to make over many lifetimes. His approach, based on logic and reason, helped to shape the modern scientific outlook.

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Louis Pasteur was born in 1822 in the French village of Dole. His father, Jean Pasteur, was a tanner of rather humble educational background. Growing up in the nearby town of Arbois, young Louis was very much interested in drawing and fishing and exhibited a remarkable talent in portrait painting. His hidden scientific genius never came through during the early days of education and he was considered a very ordinary student. As he grew older, Pasteur started developing a passion for the chemical sciences.

Laying the Foundations of Stereochemistry

The first seminal discovery that shot Pasteur to fame was made as a doctoral student in the laboratory of Antoine Balard at the École Normale Supérieure in Paris. His research problem was on tartaric acid found in natural sources such as tamarind and grapes (deposited at the bottom of wine barrels), that can also be chemically synthesized. It was already known that tartaric acid from many natural sources can rotate the plane of polarized light

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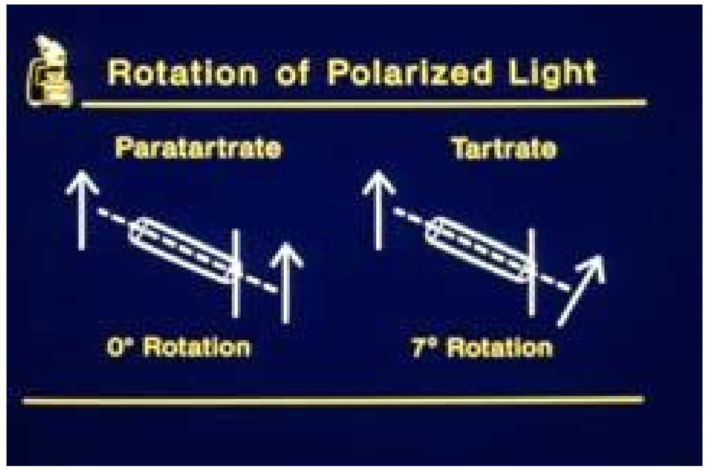
Figure 1.



to the right whereas the form known as paratartaric acid or the racemic form had no optical activity. This was strange since the chemical composition of both forms were identical. The solution came when he examined the crystals of the racemic form minutely. Pasteur could discern two types of crystals in the mixture that had different symmetries – the two forms were mirror images (see article by G Nagendrappa in this issue). Upon separation of the two types of crystals with a pair of forceps, Pasteur could demonstrate that their solutions could rotate polarized light in opposite directions. The racemic form therefore was a mixture of the two forms that rotated polarized light in opposite directions, thereby canceling out each other. Pasteur had discovered the key to stereochemistry. He was barely twenty-five years old when this seminal discovery was made.

Pasteur demonstrated that the racemic form of tartaric acid was a mixture of the two forms that rotated polarized light in opposite directions, thereby canceling out each other and thus discovered stereochemistry.

Soon after the discovery, Pasteur was offered the chemistry professorship at Dijon. By 1849, he had taken up professorship at Strasbourg and continued his work on the asymmetry of molecules. While Pasteur was in Strasbourg, he met and married the daughter of the rector of the university, Marie Laurent. Soon he left the inanimate world of chemicals and embraced the study of the living. This was prompted by a series of events that happened when he moved to the industrial town of Lille as a professor of chemistry in 1854.

Alcohol Fermentation

The town of Lille had several breweries and Pasteur was approached by one of the brewers for help with a recurring problem associated with brewing. Many times, fermentation resulted in sour tasting vinegar or lactic acid instead of alcohol. For a brewer, this was a serious problem resulting in heavy financial loss. The German cytologist and co-proponent of the cell theory of life, Theodore Schwann, had demonstrated the presence of live yeast cells in vats of wine in the late 1830s. However, any attempt to associate them with the process of fermentation was met with stiff resistance as this was a phase in chemistry when the "vital force" theory (a special force needed to support certain chemical reactions) was being exorcised. Production of alcohol from sugars by fermentation was thought to be the result of spontaneous breakdown of the sugar due to intrinsic instabilities in the molecules that could be transmitted to the fresh press by mixing it with the finished product of the previous brew.

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When Pasteur examined the different ferments that gave normal and sour brews, he made the following crucial discovery. When the brew was normal, the yeasts seen in the brew were round, plump and more importantly, uniform. All defective brews had mixtures of organisms, some round, some rod-shaped – a clear case of microbial contamination. The round yeast form turned out to be responsible for fermentation whereas the rod shaped ones were a nuisance. This he demonstrated by isolating the pure yeast form and showing that the brew was normal when it was added to the vat with fresh grape pressings. How could one prevent the brew from going bad? The brewer had to take care that the vat was not exposed to stray aerial organisms. Better still, if the finished product was moderately heated for a short time to kill any contaminating organism, the brew kept much longer, a process that came to be associated with his name – pasteurization. This is the same process that helps to keep the milk in sachets and tetrapacks from going bad. It is now applied to many other food products such as fruit juices to keep them longer without spoiling.

Spontaneous Generation

His discoveries on fermentation brought Pasteur face to face with the ongoing debate of the time on the "spontaneous generation" of life – the idea that living organisms can emerge from inanimate matter. At what point do yeasts enter the brew? Do they come from somewhere or are they generated by the brewing process? For Pasteur, it was strange that the subject of spontaneous generation was still alive in spite of the classic experiments of the Italian biologist Lazzaro Spallanzani nearly a hundred years ago and the more recent work of Schwann which showed that meat broths do not go bad if they are heated and prevented from getting contaminated by sealing the flasks. The major criticism of the work of these pioneers was that by heating, they eliminated a gaseous component in air that was necessary to facilitate spontaneous generation and by sealing the container, this was prevented from reentering.

Pasteur answered these by designing many clever experiments. To start with, he showed that the yeasts entered the brew from the skin of grapes; if the juice is extracted from inside without contacting the skin, it did not ferment. To counter the criticism of the Spallanzani experiment, Pasteur devised an ingenious solution. He heated broth and fruit juice in open flasks with narrow necks with a sharp bend (swan neck), with the tip pointing downward (*Figure* 2). The narrow and downward-pointing neck prevented dust particles from entering the flask while air circulation was permitted. The broths stayed without spoiling for many days. At the Pasteur Institute in Paris, some of the flasks still remain contamination-free after more than a century! This was the death blow to the theory of "spontaneous generation". Some historians however find fault with Pasteur for not giving sufficient credit to his predecessors, particularly Schwann, in his writings, a malady that is all too familiar in the modern day world of science!

Figure 2. Pasteur's Swan-neck Bottle.

Work on Silkworm

By 1857, Pasteur had come back to Ecole Noramle as the Director of Scientific Studies. His success with the wine industry prompted the French agricultural department to seek his help with solving the problem of silkworm disease that was having a serious impact on the silk industry in Europe. For Pasteur, fresh from his triumphs on the brewery front, the logical connection between the two problems was obvious. Diseases afflicting the silkworm must be due to microorganisms. Though he could never identify the causative agent, he could help the farmers by showing them how to isolate the healthy worms from the sick ones. The simple method was effective in arresting the spread of the disease.

Cholera and Anthrax

Pasteur had more success working with chicken cholera in 1878, though this was a lucky break. By 1876, the germ theory of disease was taking shape, based primarily on the pioneering work on anthrax by Robert Koch (see *Resonance,* Sept. 2006). It became known that chicken cholera was caused by a bacterium. Pasteur found that chicken injected with fresh cultures of the cholera bacterium died shortly. By chance, when they were injected with aged cultures, they became slightly sick, but survived. Not only that, they could remain alive and healthy even when they were among sick birds. The injection of aged cells had produced immunity. This was a momentous discovery. For a while though, Pasteur thought that he had hit upon a panacea – his injection protected the chicken from all diseases! Additional experiments showed that the protection was only against the disease associated with the specific bacterium that was injected.

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These serendipitous results prompted Pasteur to apply the same logic to anthrax. To begin with, Pasteur demonstrated that he could culture the anthrax bacillus from dilute suspensions of blood from infected animals. Could injections of the "attenuated" strains of anthrax protect animals from infections? Though the injections did fail occasionally, there was a fair amount of success with his idea of anthrax vaccination. In the famous public experiment conducted at the French village Pouilly le Fort in 1881, twenty four sheep were inoculated and an equal number not injected (the control group). While every single one of the inoculated sheep survived, all the control sheep died. In spite of this tremendous public success, many including Koch believed that Pasteur was rather premature in his vaccine preparation and this was the main bone of contention between the two. Koch was justifiably angry with Pasteur for not giving him sufficient credit for his anthrax work.

Pasteur's Swansong – The Rabies Vaccine

Though he was sixty years old and not in the best of health, in 1882, Pasteur embarked on probably his most important study, on rabies. Known as hydrophobia, rabies was invariably fatal and the patient usually died tragically. His experience with anthrax and cholera suggested to him that a germ was involved in this case also. However, the blood or saliva of infected animals never showed specific bacteria that could be associated with the disease. In Pasteur's view, it was still a germ though invisible. We know today that rabies is caused by a virus that is too small to be observable under a light microscope.

Usually there is a delay between the bite by a mad dog and the development of the rabies symptoms that are normally associated with disturbances in brain and motor functions. From this, Pasteur deduced that the germ had to reach the brain through the peripheral nervous system and the spinal chord. Though there was a definite risk to his life and that of his colleagues, Pasteur started working with infected animals. As in the case of chicken cholera, he was rewarded by a great stroke of luck. One of the rabid dogs in his laboratory surprisingly survived the disease. This was almost unheard of. Not only that, when injected with extracts of brain tissue derived from rabid animals, the dog remained healthy. The previous bite, resulting probably

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in a sub-lethal infection, had given immunity to the dog. Charged by this observation, Pasteur and his colleague Emil Roux continued the work with renewed vigour. Spinal chord extracts taken from infected rabbits were dried and kept for several days before injecting into experimental dogs. Dried extracts kept for more than two weeks produced no illness upon injection. More importantly, even after injection with fresh extracts, these animals survived. Pasteur and Roux had discovered a rabies vaccine.

Pasteur and Roux had discovered a rabies vaccine.

This time, Pasteur was more circumspect and cautious compared to his earlier foray into anthrax vaccine. Age and experience had probably mellowed him. Though he was sure that the attenuated brain and spinal tissue preparations had the potency to immunize individuals against rabies, he was not yet ready for human trials. Again, this was brought about by a chance event. A nine year old French boy, Joseph Meister, was severely bitten by a rabid dog, and his mother implored Pasteur to save his life. Moved by her pleas and the recommendations of physicians who were aware of his work, Pasteur started the two week long treatment, injecting the boy with extracts of increasing potency. This required great mental strength on his part as he had not undertaken a human trial before. Little Joseph Meister survived and worked as the gatekeeper of the Pasteur Institute for many years.[1] History had been created. Pasteur's name had become a legend and patients from all over Europe flocked to him for treatment, even though he was not a physician.

[1] It is ironic that forty five years after this historic event, Joseph Meister took his own life rather than comply with the occupying Nazis who ordered him to open Pasteur's crypt at the Pasteur Institute.

Epilogue

All through his brilliant career, Pasteur had earned the awe and respect of the world scientific community. After the success of the rabies vaccine, public money was raised to build the Pasteur Institute, primarily for the treatment of rabies. Sadly though, the rabies vaccine was to be the last of Pasteur's triumphs. With ailing health, he found it increasingly difficult to work and successive strokes left him weaker by the day. At the age of seventy, he was honoured by the French Republic with a special

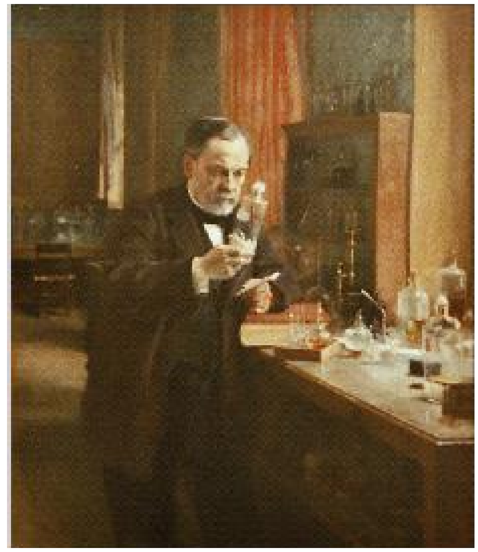

Figure 3. Pasteur in his laboratory. (Portrait by Albert Edelfelt, 1895)

medal. Pasteur barely managed to attend the function. In 1895, he was awarded the Leeuwenhoek Medal, the highest honour for a microbiologist. He died the same year in the arms of his devoted wife. A devout Catholic throughout his life, Pasteur was given a funeral befitting a national hero and was buried in the Notre Dame Cathedral. His mortal remains were later transferred to a crypt at the Pasteur Institute.

In spite of some of his limitations such as a passionate and short tempered nature, egoism and reluctance to give credit to predecessors and contemporaries, Pasteur will be remembered as one of the finest scientific minds the world has seen. These limitations pale in comparison to the significant contributions that he made to human welfare.

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Suggested Reading

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