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Ramón y Cajal, microbiologist

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In early January 1884, Santiago Ramón y Cajal (1852–1934) (Fig. 1) moved to Valencia to take up the chair of Anatomy at the University of Valencia. He was full of enthusiasm to carry on with the histology studies he had recently begun in Zaragoza with the help of only a modest Verick microscope bought with his savings from his work as a medical captain in Cuba and a barber's razor as a microtome. He and his wife liked Valencia. He described it like this: "I found myself in a new country, with a very mild climate, with fields full of blooming agaves and orange trees, with people in whose spirit nested courtesy, culture and talent. No wonder Valencia is called the Athens of Spain."

Ramón y Cajal wanted to carry out micrographic studies as well as become involved in the social life of the city; he wanted to meet cultured people and to be able to practice photography and chess, hobbies, he said, "where you do not bet money but your brain, our greatest capital asset." He joined the Casino de Agricultura and the Ateneo Valenciano. The latter was a scientific-literary club where the cream of Valencia's youth used to gather. His salary as a university professor was poor so for a little extra income he set up a small laboratory in his own house where he gave Histology and Pathological Histology lab lessons. His students were physicians who were either preparing their doctorates or anxious to acquaint themselves with microscopy and bacteriological anatomy, a promising new science that had sprung from the brilliant discoveries made by Louis Pasteur and Robert Koch.

The 1880s were years of major achievements in the knowledge of infectious diseases. The French school, led by Pasteur, and the German, led by Koch, laid the foundations of bacteriology, which developed dramatically over those years and the first decade of the 20th century. By



Fig. 1. Santiago Ramón y Cajal (1852–1934), Nobel Laureate in Physiology or Medicine, 1906

The author, grandson of Ramón y Cajal, wrote this text as the Preface to the facsimile published on the occasion of the "Cajal on consciousness" Congress held in Zaragoza (Nov. 29–Dec. 1, 1999) to commemorate the centennial of the publication of "Textura del sistema nervioso del hombre y de los vertebrados". The book includes the monograph "Studies on the cholera virgule microbe and prophylactic inoculations" written by Ramón y Cajal for the County Council of Zaragoza.

1880 Pasteur had already obtained an effective vaccine against ovine anthrax and had studied puerperal fever. From 1881 to 1885, he worked on the study of rabies and how to prevent it. He was able to artificially obtain less virulent strains of the causal agent of rabies, which had lost their pathogenic strength but retained their capacity to stimulate an immunological response in a host. This achievement led to a vaccine against rabies that he applied to a boy called Joseph Meister, saving the child's life. Over the next fifteen months, 2,500 people were vaccinated against rabies. By inoculating rats with the anthrax bacillus, Robert Koch, founder of the German school of bacteriology, had demonstrated for the first time the transmission mechanism of infectious diseases caused by bacteria. In 1879 he discovered the gonococcus which caused blennorrhoea, and in 1880 the rod-shaped bacilli which caused typhoid fever and leprosy, as well as the protozoan that causes malaria. In 1882 he discovered the bacillus that caused tuberculosis, and while studying an outbreak of cholera in Egypt, he identified the "comma-shaped" bacillus, also known as *Vibrio cholerae*. Pupils of Pasteur's in France and of Koch's in Germany began pursuing bacteriological studies, which led to the early discovery of microorganisms causing erysipelas, tetanus, diphtheria, pneumonia, meningitis, bubonic plague, etc. In 1882, Ilya Ilych Metchnikoff (1845–1916) produced his theory of cellular immunity, based on the phagocytic capacity of the macrophage, and in 1888 Charles Robert Richet (1850–1935) discovered the role of immunity in the infection process.

This was the flourishing state-of-the-arts of infectious diseases when, in 1885, a cholera epidemic broke out in the city of Valencia and the surrounding region. The epidemic spread throughout Spain and caused terrible havoc among the population. Ramón y Cajal felt compelled to give up his studies on cell biology and histology, and devoted himself entirely to the study of the *Vibrio cholerae*, recently discovered by Koch. He soon set up culture media and ordered incubators to grow the bacterium. Hospitals were overcrowded with infected people and much controversy arose among Valencian physicians regarding the remedies to apply. Older doctors could hardly believe in a microbial origin of the disease, and prescribed Sydenham's laudanum. Younger physicians, on the contrary, more open to medical progress, were convinced of a bacteriological etiology. They advised the population to boil drinking water and to cook all food as an effective prevention measure. In his Memoirs, Ramón y Cajal writes: "Those were days of intense emotion; the population, decimated by the calamity, lived in a state of anxiety but never lost their calm."

At this point, the well-known Catalan physician Jaime Ferrán arrived in Valencia claiming the effectiveness of his new anti-cholera vaccine. Ferrán had acquired experience with cholera during an epidemic in southern France in 1884. Based on his observations, when he had tried to produce

experimental cholera in laboratory animals, he thought he had produced a vaccine that would cause immunity after the injection of live germs. Ferrán suggested that massive vaccination should be carried out to prevent the epidemic from spreading. His proposal provoked a heated controversy that started in Valencia and soon spread to other parts. The younger physicians passionately sided with Ferrán. Finally, the authorities allowed Ferrán to go ahead with his vaccination campaign, and more than 50,000 people were vaccinated. Initially, Ramón y Cajal collaborated with Ferrán by supplying him with microbial samples for his studies. Later, he decided, however, to maintain his own independent criterion and to safeguard himself from any suspicion of economic profit which might result from the massive vaccination. Ferrán, on the other hand, always kept the elaboration of his vaccine secret.

The County Council (Diputación) of the province of Zaragoza asked Ramón y Cajal and Dr. Lite, an official delegate, to write up a report on the cholera epidemic that had struck the Valencia region, and on the value of prophylaxis claimed by Dr. Ferrán. During the summer of 1885, when cholera had spread to Aragón and most of Spain, Ramón y Cajal and his family moved to Zaragoza. After his lecture at the County Council describing his observations and the experiments carried out in Valencia, Ramón y Cajal decided to go on with his research. He settled in his father's country estate in San Juan, called Torre de Los Canales where he set up an improvised laboratory. There he inoculated guinea pigs and rabbits with samples from virgule cholera cultures. In the meantime, the disease had spread to the city of Zaragoza and the surrounding countryside. Ramón y Cajal noticed that the immunization of inoculated animals was more complex than he had expected. After repeated subcutaneous injections of the cholera vibrio, he found some kind of resistance to the infection in the laboratory animals when they were inoculated with higher doses of the microorganism. But the response could be studied only on the surface, at the very place of the injection, as it was impossible to reproduce an intestinal infection similar to that suffered by humans in these animals. Therefore, it was impossible to obtain conclusive evidence that the inoculation of the microbe would immunize against the disease. From his experiments, Ramón y Cajal concluded that the vaccine proposed by Ferrán was of little effect.

At the end of September 1885, Ramón y Cajal wrote a report entitled "Studies on the cholera virgule microbe and prophylactic inoculations", which was published by the County Council. It was a monograph illustrated with drawings by the author himself, some in full color. Ramón y Cajal's report confirmed the observations by Koch and Ferrán on the causal agent of cholera. The report also included original technical observations, such as new methods to stain and grow microbes colonies, as well as a description of the degenerating and regressive forms of the comma-shaped

bacillus, which was a mere consequence of the deterioration of nutrients in the culture medium. Some of Ramón y Cajal's major contributions in that report were: (a) to prove the limited infectious capacity of the cholera vibrio when an animal was inoculated by subcutaneous injection, (b) the strong virulence in an intraperitoneal inoculation, and (c) the possibility of avoiding the toxic effects of the microbe when certain quantities of the cholera-germ culture, killed by heat, had been previously injected hypodermically. In this way, in 1885, Ramón y Cajal had introduced for the first time the concept of a chemical vaccine, one year before the American researchers Salmon and Smith described the advantage of vaccinating animals by inoculating them with dead bacteria. Ramón y Cajal commented: "Needless to say, these modest theoretical-experimental contributions went unnoticed by bacteriologists in Paris and Berlin. Those were indeed very hard times for Spanish researchers. We had to fight against both the universal prejudice of our lack of culture, and our radical indifference towards great biological problems."

Ramón y Cajal's criticism of the prophylaxis postulated by Ferrán interfered with their mutual friendship. Two years later the two men coincided in Barcelona: Ferrán as Director of the Municipal Microbiology Laboratory; Ramón y Cajal as Professor of Histology at the School of Medicine of the University of Barcelona. In 1906, Ferrán was dismissed from his official post, whereas Ramón y Cajal was awarded the Nobel Prize on Physiology or Medicine.

"Despite everything, although my work did not produce a response in the laboratories of Paris and Berlin, which was neither here nor there, it was a material and spiritual reward of great transcendence for my career. The authorities of the County Council of Zaragoza were so grateful for the zeal and objectiveness with which I had worked for them that they presented me with a superb Zeiss microscope." Indeed, the County Council bought him a microscope with a 1.18 objective-lens of homogeneous immersion, which was the best light microscope available at the time. Ramón y Cajal's close relationship with bacteriology at the time made him wonder whether it would not be convenient to carry on with the study of microbes but bacteriology was a luxurious science which required expensive laboratory equipment and plenty of animals for research. Histology, on the other hand, was an inexpensive, modest field of science; once you had a microscope and a microtome, it only required the replacement of some reagents and the sacrifice of a few mice or rabbits. "I have often asked myself if it would not have

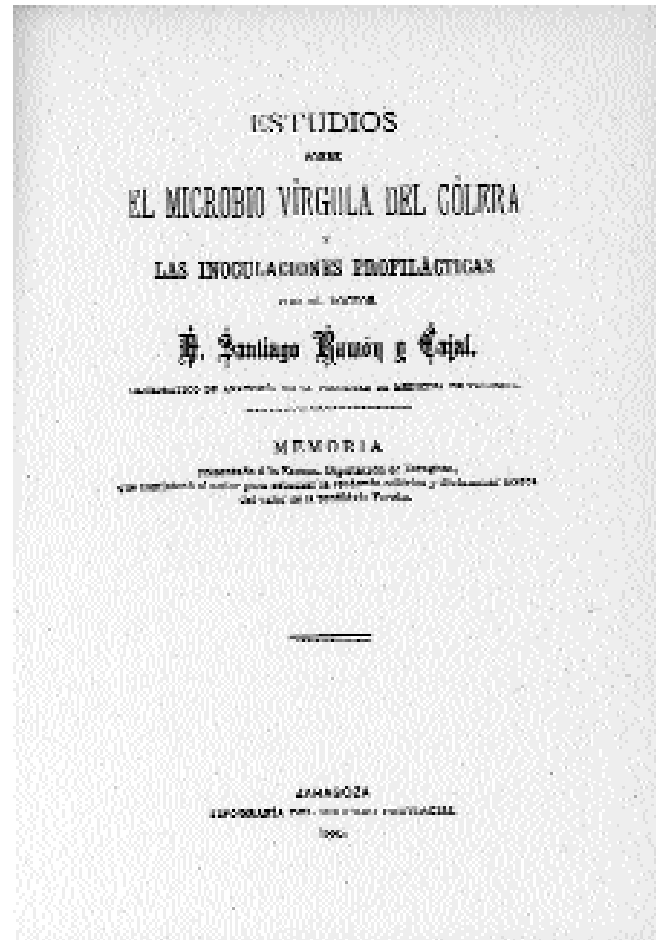


Fig. 2. Facsimile of the report written by Ramón y Cajal for the County Council of Zaragoza, which "had commissioned the author to study the epidemic of cholera and to issue a report on the value of Ferrán's prophylaxis". The monograph, reproduced in November 1999, was originally published by Tipografía del Hospicio Provincial in 1885

been better for my spirit and my economy to have followed the trend, replacing, as so many did, the cell for the microbe."

When Ramón y Cajal returned to Valencia in 1885, he had already decided to pursue Histology, "that of quiet pleasures". The study of cells and tissues kept him busy for the rest of his life. The County Council of Zaragoza has fortunately republished his monograph (Fig. 2) on the cholera virgule, forgotten and very difficult to find nowadays. And in this way, they wish to pay homage to Ramón y Cajal for his excellent work .