RETROSPECTIVE

Christian de Duve: Explorer of the cell who discovered new organelles by using a centrifuge

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Christian de Duve, whose laboratory in Louvain discovered lysosomes in 1955 and defined peroxisomes in 1965, died at his home in Nethen, Belgium at the age of 95, on May 4, 2013. De Duve was the last of a group of eminent physiological chemists who, by the 1940s and 1950s, began to explore the subcellular organization of biochemical pathways and thus forged the emergence of Modern Cell Biology. Christian De Duve, Albert Claude, and George Palade received the Nobel Prize in 1974 "for their discoveries concerning the structural and functional organization of the cell."

De Duve was born on October 2, 1917 in Thames Ditton, United Kingdom, a town not far from London where his family had sought refuge during World War I. After a classic education in a Jesuit school in Antwerp, De Duve entered the Medical School of the Catholic University of Louvain in 1934, with no intention of becoming a scientist. He credited a student apprenticeship with Joseph Bouckaert, who headed the physiology laboratory, for sparking his interest in basic research. A major concern of Bouckaert's research was the mechanism of action of insulin. De Duve participated in experiments in which rather crude preparation of the hormone were administered to hepatectomized animals, which led him to adopt the idea that insulin acted primarily on the liver, and for many years he investigated with intensity the validity of this notion.

De Duve was in his last year of medical school when the Germans invaded Belgium in 1940. His involvement in the war was minor, as he was drafted as a medic, and soon was able to return to Louvain to finish medical school. However, by that time de Duve's commitment to research was too strong for him to pursue a career in medicine. After completing a Master's thesis in chemistry at Louvain in 1946, de Duve spent over a year as a postdoctoral fellow in Stockholm with Hugo Theorell, a pioneer in the study of oxidizing enzymes who received the Nobel Prize in 1955. Theorell's laboratory provided an ideal place for de Duve to learn the most advanced tools of enzymology, which were central to his later work. His Swedish sojourn was followed by a visit to the laboratory of Carl and Gerty Cori in St. Louis, the Mecca of carbohydrate research at the time, where he worked for a few months with Earl Sutherland, with whom he identified glucagon as a contaminant of insulin preparations widely used in those days. Glucagon was often referred to as the "hyperglycemic glycogenolytic factor" and de Duve later proudly referred to this work as his "re-discovery of glucagon." Sutherland's further work on the hormonal control of glycogenolysis led him to the discovery of cAMP, for which he received the Nobel Prize in 1971.

In 1948, de Duve returned to Louvain, where he intended to pursue his interest in carbohydrate metabolism and the action of insulin. With a newly assembled group of young collaborators, de Duve decided to characterize the hexose phosphatase, which—following the action of phosphorylase on glycogen—was responsible for the unique property of the liver to release glucose into the blood. The researchers identified a liver phosphatase specific for glucose-6-phosphate and correctly concluded that it was responsible for that effect. Their subsequent attempts to purify that enzyme set them on the track to the discovery of lysosomes.

De Duve and his group observed that an acidic pH caused an irreversible precipitation of the glucose-6-phosphatase, which led de Duve to infer that the enzyme could be associated with agglutinated cytoplasmic membranes. Hence, the group decided to follow the distribution of the enzyme in the various cell fractions that could be obtained from liver homogenates by a procedure developed by Claude, which used mild homogenization conditions and was designed to preserve the integrity of subcellular organelles.

It was most fortunate that in the course of these experiments, in addition to following the distribution of glucose-6-phosphatase—which was found to be primarily in the small granule fraction called "microsomes" by Claude—de Duve's group also followed, as a control, the distribution and activity in the subcellular fractions of acid phosphatase, an enzyme with an optimum pH of 5 and a very broad substrate specificity, which is found in almost all tissues. Because this enzyme was soluble when homogenates were prepared in a Waring blender, the researchers expected to find it in the final supernatant obtained by Claude's procedure. However, the activity was found to be present to various extents in all of the fractions and, in particular, in the large granule fraction known to contain the mitochondria. This finding was puzzling, as were also the facts that the sum of the activities in all of the fractions was much greater than the activity in the whole homogenate, whose activity was much lower than when the Waring blender was used for the homogenization. These intriguing observations were obtained in December 1949 just before a weekend, and could have discouraged de Duve's group from further studies on acid phosphatase, an enzyme that, after all, was not of major interest to them and

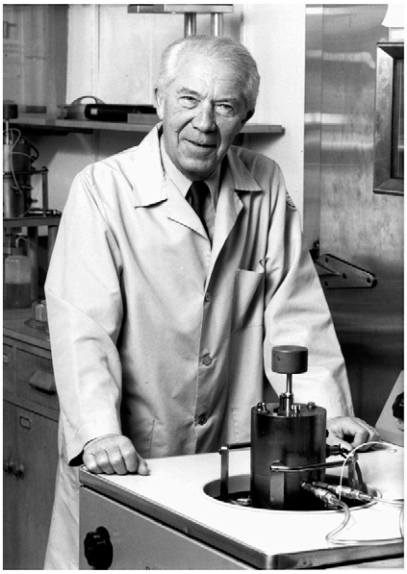

Photo courtesy of The Rockefeller University.

Christian de Duve.

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www.pnas.org/cgi/doi/10.1073/pnas.1312084110

had been chosen as a control. It seems serendipitous that they nevertheless decided to store the samples in the refrigerator and reassay them at a later time. The results obtained five days later came to steer the researchers onto a new path that led them to their discovery, first of the lysosome and later the peroxisome.

De Duve and his group found that, with the exception of the activity in the final supernatant, acid phosphatase activities had risen proportionately in all of the fractions, as well as in the unprocessed homogenate, whose activity now corresponded to the sum of the activities in all of the fractions. They soon showed that the effect of "ageing" the fractions in the refrigerator could be recreated by treatments that disrupt membranes, such as blender homogenization or repeated cycles of freeze-thawing. On this basis, de Duve insightfully concluded that the "latent enzyme" was sequestered within "membrane sacs" that made it inaccessible to the substrates.

The studies on acid phosphatase prompted de Duve's group to develop a procedure that separated from the fraction rich in mitochondria a "light mitochondrial fraction" or L fraction, which contained most of the acid phosphatase but very little cytochrome oxidase activity. What de Duve's laboratory, in fact, accomplished was the purification of a new organelle solely on the basis of analytical biochemical procedures, guided by measurements of specific enzymatic activities, which are now regarded as "marker enzymes." The finding that four other acid hydrolases—β-glucuronidase, cathepsin D, ribonuclease, and DNAse—displayed latency and were also enriched in the L fraction led de Duve to formulate the "lysosome" concept: that is, a membrane-bounded organelle that contains acid hydrolases with various specificities and whose main function is the intracellular digestion of macromolecules. Later, as progress was being made in elucidating the broad function of lysosomes, De Duve also coined the terms "endocytosis," "phagocytosis," and "autophagy" to designate pathways that bring substrates for digestion in lysosomes and, today, are active fields of research in cell biology.

Remarkably, de Duve arrived at the lysosome concept without resorting to any microscopic examination of his samples. In fact, there was no microscope in his laboratory and he entitled his Nobel lecture "Exploring Cells with a Centrifuge." The lysosome obtained a morphological identity in 1955 as a result of a brief collaboration with Alex Novikoff, a visiting scientist from the Albert Einstein College of Medicine in New York, who had expertise in electron microscopy. Novikoff's micrographs showed that the "light mitochondrial" fraction contained membrane bounded "dense bodies" similar to those present in the peri-canalicular region of hepatocytes.

The discovery of the lysosome inaugurated a new era in cellular physiology and pathophysiology, which was followed by the identification, first in Louvain and then throughout the world, of more than 40 lysosomal storage diseases resulting from mutations in genes for specific hydrolases.

The first inkling that, in addition to lysosomes, the light mitochondrial fraction also harbored an as yet unknown organelle, was the finding that urate oxidase—an enzyme that is not an acid hydrolase and does not show latency—had a similar distribution in subcellular fractions as acid phosphatase. By 1960, de Duve had found that this was also true for catalase and for D-amino acid oxidase, then thought to be mitochondrial enzymes. He later extended these findings to several other peroxide-producing oxidases with a sedimentation behavior similar to catalase, an enzyme that breaks down their product. De Duve had the insight that a functional linkage between these enzymes existed, which was made possible by their inclusion in the same particle. Thus, the concept of a peroxisome was being born, but it was not to be presented publicly until several years later, after de Duve had begun to split his time between Louvain and New York.

In 1962 de Duve accepted an attractive offer to create and direct a laboratory at The Rockefeller Institute in New York, while maintaining his laboratory in Louvain. He was able to transfer to his new laboratory the various technologies developed in Louvain by arranging for regular visits of his major Belgian associates to New York. In both laboratories, de Duve continued the characterization of the newly discovered oxidase-containing particles first identified in rat liver. Three years later, only after particles with a similar sedimentation behavior and biochemical properties were found in rat kidney and in the ciliated protozoan *Tetrahymena pyriformis*, did he announce, at a meeting of the American Society of Cell Biology, that he had discovered a new organelle, for which he proposed the name "peroxisome."

Again in this case, electron microscopy showed that, morphologically, the new organelle corresponded to membrane-bounded particles of unknown function that had been recognized by microscopists to be present in almost all tissues and had been designated "microbodies."

Subsequent studies from many laboratories, including those from de Duve's and his former associates and students, showed that peroxisomes—first discovered in mammalian tissues, where they play important metabolic roles, including the β-oxidation of very long-chain fatty acids by a pathway different from that in mitochondria—are members of a large family of evolutionarily related organelles present in many different eukaryotic cell types and organisms, including plants, and protozoa, where they carry out distinct functions and have been given specific names, such as glyoxysomes and glycosomes. Thus, with his discovery of peroxisomes, de Duve once more laid the foundation for the growth of a new chapter in the burgeoning field of Cell Biology.

In 1974, soon after receiving the Nobel Prize, de Duve, inspired by his experience at The Rockefeller Institute, championed the creation in Brussels of a new multidisciplinary "International Institute of Cellular and Molecular Pathology," with a translational mission, which he originally directed and at his 80th birthday was renamed the "de Duve Institute."

De Duve left a major imprint in the biological sciences through the work he carried out on both sides of the Atlantic and through the many scientists who trained with him. He was a highly cultured person who spoke four languages fluently and wrote elegant prose in at least two of them. De Duve's interests extended well beyond the areas of his scientific contributions, into the realms of philosophy, the theory of knowledge, the origin of life, and the evolution of the eukaryotic cell. He published extensively his thoughts on questions from almost all these fields, in lucid articles as well as in books. De Duve also wrote many engaging historical accounts of the major scientific discoveries made in his laboratories and in all of them he took great care to give credit to his younger associates and to point out their specific contributions.

Christian de Duve was a warm colleague and a fascinating conversationalist. Those of us who had the good fortune of knowing him personally will sorely miss him.