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An Unconventional Traveler: [Dr. Christian de Duve]

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RESEARCH PROFILES

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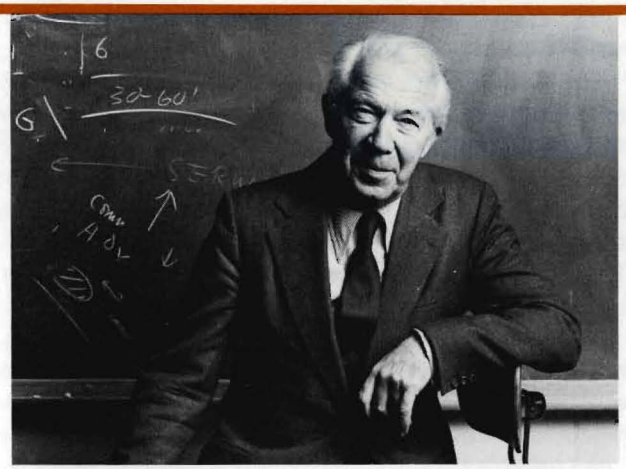
Suddenly, right in front of us, looms a dark, ungainly blimp...Collision is inevitable...hurling us into what turns out to be a thoroughly unpleasant environment. Everywhere we look are scenes of destruction: maimed molecules of various kinds, shapeless debris, half-recognizable pieces of bacteria and viruses, fragments of mitochondria, membrane whorls, damaged ribosomes, all in the process of dissolving away before our very eyes.

from "A Guided Tour of the Living Cell"
CHRISTIAN DE DUVE, 1984

An Unconventional Traveler

In his Nobel Lecture, Christian de Duve, Andrew W. Mellon Professor of The Rockefeller University, likened himself to a character in a tale who "traveled the whole of Sweden, from Skåne to Lappland, on the wings of a white gander. I, too," he said, "have made a wonderful journey, using, like Nils Holgersson, an unconventional mode of travel." For thirty-five years Dr. de Duve has roamed the living cell. Unlike Nils Holgersson's, the landscape he has traversed was largely uncharted when he set out.

For a century following the recognition of the cell as the basic unit of life, biologists, bumping always against the limits of the light microscope, could distinguish within the cellular border only a nucleus and some shadowy forms surrounded by a mysterious jelly. Then, in 1944, Albert Claude, a Belgian scientist working at what was then The Rockefeller Institute for Medical Research, in collaboration with Keith R. Porter and Ernest F. Fullam, published the first electron micrograph of a cell. "The structural complexities that were revealed," says Dr. de Duve, "exceeded anything that had been imagined." Two years later, Claude published two papers collectively titled "Fractionation of Mammalian Liver Cells by Differential Centrifugation," in which he described a method for separating cellular structures intact, making them accessible for study of their biological activity. The age of modern cell biology had begun.



Christian de Duve

The studies that began with Claude, who died last year in Belgium at the age of eighty-four, yielded, within one generation, an essentially complete catalogue of the cell's working parts, achieved by a small band of pioneers who refined and extended his techniques. Among them was George Palade, now at Yale University, who spent twenty-eight years at Rockefeller, the early ones with Claude. During that time, working with the electron microscope and fractionation techniques, he isolated and explained mitochondria, the cell's energy-converting power plants, and discovered ribosomes, the protein "factories."

Another explorer was Christian de Duve, a Belgian like Claude, who was lured into cell biology by "a chance observation." The passage at the opening of this profile is from a book by Dr. de Duve published this summer by Scientific American

Miklós Müller

Books, Inc., in collaboration with The Rockefeller University Press. The "unpleasant environment" it describes is a lysosome. Central to cellular function, lysosomes are minuscule sacs – organelles – filled with powerful enzymes that break down the substances ingested by cells, performing the critical tasks of digestion and waste disposal. Lysosomes were identified by Dr. de Duve, at the Catholic University of Louvain, in 1955.

In 1974 Albert Claude, Christian de Duve, and George Palade shared the Nobel Prize in Physiology or Medicine for their "discoveries concerning the structural and functional organization of cells."

In his Rockefeller laboratory, which he established in 1962, reflecting on the events that led him to lysosomes, Dr. de Duve smiles and confides, "I wasn't attracted to science as a boy. Although I thought medicine would be a rewarding career, I dreaded the ordeal of chemistry and biology. But everything changed when I entered medical school at Louvain in 1934. The head of physiology there, Joseph Bouckaert, was a true scientist. I walked into his laboratory and I was hooked.

"I began by working with a group studying insulin. I became so involved I almost didn't finish my clinical training. I even made a discovery, or, rather, a rediscovery; I found glucagon, an insulin antagonist, as a contaminant of Lilly insulin. I had unearthed this 'impurity' – it is really an important hormone – in the old literature, where it had been buried after crystalline insulin was found to be free of it. I came to the conclusion that glucagon must have reappeared surreptitiously as a result of a change in the procedure for purifying insulin. This, at least, was the only way I could account for a major contradiction between my own experimental results and much of the recent American literature. By then, however, the war had started and I couldn't test my hypothesis. The day my country was liberated – well, no, the second day, the first day, of course, everybody got drunk – I went to an American army post and got some Lilly insulin. We checked it in the lab and found that it did indeed contain glucagon. I felt like Le Verrier when he discovered Neptune."

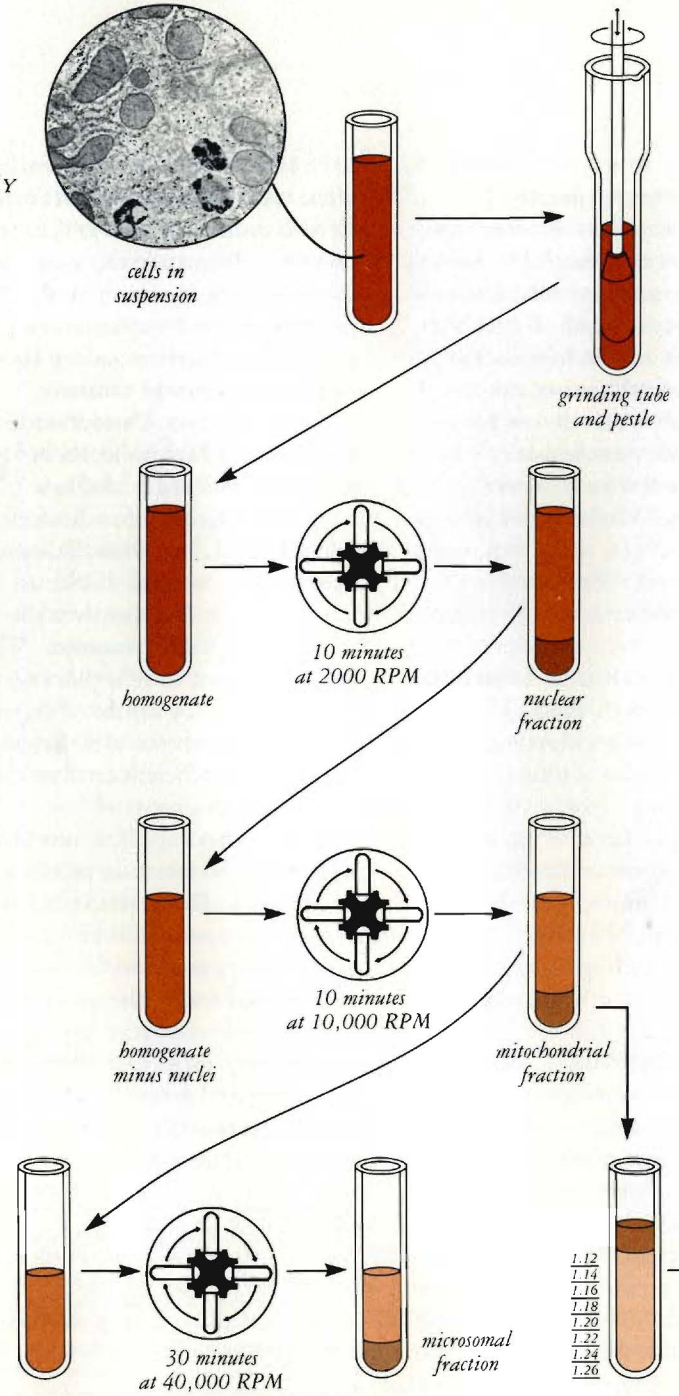
After the war, convinced that chemistry rather than physiology was the path to his goal, which was to elucidate the mechanisms of insulin action in the liver, Dr. de Duve, who by then had added a chemical degree to his medical degree, went to Stockholm to study with "the coming man in biochemistry," Hugo Theorell. Two years later, equipped with "the tools of the trade," he went to Washington University in St. Louis. "Carl and Gerty Cori were then the big experts in carbohydrate metabolism," he says, "and I asked if they would let me work with them for six months before I had to be back in Louvain, where I had just received an appointment." At Washington University, he and Earl Sutherland showed that glucagon is made by the alpha cells of the islets of Langerhans in the pancreas and is presumably a hormone. "It was a nice piece of work. I've been very lucky in picking teachers." (Carl and Gerty Cori won a Nobel Prize in 1947, Theorell in 1955, and Sutherland in 1971.)

PECULIAR ENZYMES AND DENSE BODIES

Back at Louvain, working with Géry Hers, his first student and continuing colleague, Dr. de Duve set up a small biochemistry laboratory and started a study of an enzyme that was assumed to be involved in insulin action. Enzymes are proteins that speed up or otherwise modify biological activity. In the course of trying to purify the enzyme glucose 6-phosphatase, they kept losing it. When they made the medium more acid to get the enzyme out of solution, they couldn't get it back in solution again by neutralizing the acid, which, says Dr. de Duve, was not normal behavior for a soluble protein.

Earlier, on his way home from St. Louis, Dr. de Duve had stopped off in New York to pay his respects to his countryman Albert Claude, who gave him copies of the papers on cell fractionation. Reading them in Louvain, he thought he saw a clue to the mystery of the missing enzyme. As he explains: "Claude had shown that cell particles will agglutinate at the acid level we had exposed our enzyme to. It occurred to me that perhaps the enzyme might be bound to a structure. I decided to try Claude's methods to see if I could find out where it was." At that point he also was able to seek some advice from Claude himself, who had shortly before returned to Belgium to direct the Bordet Institute.

Separating Cell Structures By Centrifugation



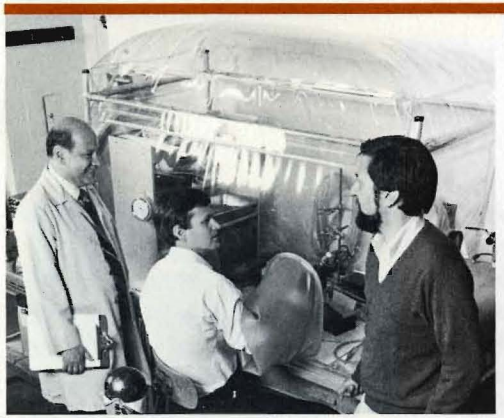
In the process of differential centrifugation, cells are ground in a solution and the resulting homogenate (misnamed because it is anything but homogeneous) is spun in a centrifuge at progressively higher revolutions per minute to separate different particles. In a final step, called density-gradient centrifugation, crude fractions containing mostly mitochondria are subjected to very high centrifugal force in a medium of graded density which separates the mitochondria, lysosomes, and peroxisomes.

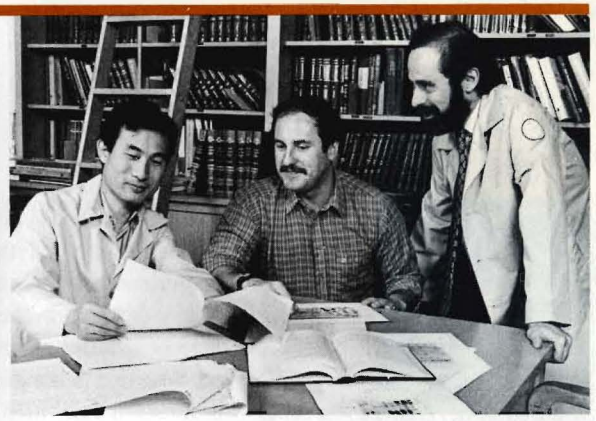
Up to that time, the methods used by biochemists to extract cells usually broke the more fragile intracellular structures. Claude had found a way to disrupt cells gently in a liquid medium to yield cell preparations still containing unbroken, functional organelles. The suspension, or homogenate, was then spun in centrifuges at successively higher speeds to separate and isolate the particles by size and density.

Dr. de Duve found his enzyme bound to structures called microsomes, which are fragments of internal cell membranes. Glucose 6-phosphatase was one of the first enzymes to be localized, and the first in microsomes, for which it is still the marker. Meanwhile, during the hunt, he observed another enzyme behaving peculiarly. This was the chance observation, in 1949, that would induce him to abandon insulin in favor of cell biology.

"While looking for glucose 6-phosphatase," he explains, "we also systematically measured another enzyme, called acid phosphatase. This enzyme held no interest for me per se but it had to be checked because it could give false positives. To our surprise, its activity was a tenth of what we expected on the basis of previous assays." Curious, he resisted the temptation to discard these results as a technical error. He decided to go after acid phosphatase. Its trail ended in the cell organelles that he named lysosomes.

The discovery of lysosomes depended on the centrifuge, Dr.





de Duve's "white gander," and on biochemical identification marks, because lysosomes do not have consistent structural characteristics like most other organelles. There can be hundreds of lysosomes within a single cell, possessing a bewildering array of sizes and shapes depending on the kind and amount of material they have ingested and on the stage of the digesting (lysing) process. The positive identification of lysosomes took years of research, years during which Dr. de Duve postulated and proved a number of theories concerning enzyme distribution and he and his lab mates studied the centrifugation process intensively and developed innovative applications and designs.

While Dr. de Duve was centrifuging, other scientists were improving the infant technology of the electron microscope. In 1955, with the help of Alex Novikoff of the Albert Einstein College of Medicine, Dr. de Duve obtained the first electron micrographs of a cell fraction containing lysosomes and, thereby, his first glimpse of what his "children" looked like. In the liver, they corresponded to the "dense bodies" described by microscopists. Later, using the same biochemical approach, he and his colleagues were able to isolate and characterize a different kind of organelle, the peroxisome, which they subsequently identified with the entities named microbodies by the electron microscopist who first saw them. These organelles form the basis of much of the current research in Dr. de Duve's Rockefeller laboratory.

AIR BRIDGE TO MECCA

The problems Dr. de Duve grappled with in the 1950s were not solely intellectual. The main business at Louvain was the training of physicians, and support for basic science in those years was very limited. As a member of the medical faculty, he taught hundreds of students each year and gave hundreds of exams, while directing a growing laboratory for which he was expected to raise the bulk of the funds. "Scientific papers I wrote evenings," he remembers, "in a house with four young children. I was a one-man band."

From time to time he attended scientific meetings in the United States and would revisit Rockefeller. "It was the place where most of the major work in my field was being done," he says. "To me it was Mecca. By then, the institute had become a graduate university and Detlev Bronk, an extraordinary man, was president. Palade was working with Philip Siekevitz doing the definitive studies in protein synthesis, James Hirsch and Zanvil Cohn were doing the most important work anywhere on lysosomes in immune cells, Stanford Moore and William Stein were developing amino acid analysis of enzymes. It was easy to see the reasons for the University's success: excellent people free to pursue their interests."

On one of his visits, Dr. de Duve remarked to George Palade that he would love to work at Rockefeller. "Palade, I remember, just looked at me and said, 'Are you serious?' To my utter surprise, not long after I returned home Bronk flew over to Belgium and offered me a job. Well, Louvain wasn't too happy about that, so we worked out a compromise. I would 'temporarily' run both labs, at Rockefeller and Louvain. I'm still doing it! I have a very patient wife, who also happens to love New York. Over the years there has been an air bridge, with Louvain people coming to Rockefeller and my Rockefeller colleagues and students going to Belgium. Altogether, a very rich exchange."

FIRST PHOTO:
Drs. Miklós Müller, left, Thomas Gorrell, seated, and Martin Weiss at the anaerobic chamber. Anaerobes are organisms that live in the absence of oxygen. Among them are trichomonads, single-celled protozoa in which Dr. Müller identified structures called hydrogenosomes.

SECOND PHOTO:
Dr. de Duve and longtime assistant Armando Pelaschier at the Beaufay centrifuge, a fully automated instrument designed by Dr. Henri Beaufay in Dr. de Duve's laboratory in Belgium and built at Rockefeller.

THIRD PHOTO:
Drs. Paul Lazarow, right, Yukio Fujiki, left, and Richard Rachubinski discuss new findings on peroxisomes, cell structures first described in Dr. de Duve's laboratory.

Carl Beyer, who began his Rockefeller career as a doctoral student. His current research centers on the biochemistry and cell biology of lymphocytes, white cells of the immune-defense system.

Margaret Perkins holds a laboratory dish containing red blood cells infected with malaria parasites. She has identified the proteins on the blood cells to which the parasites attach and is now characterizing the proteins on the parasite cell surface involved in the binding process.

THE COMMON THREAD

The discovery of lysosomes was followed by the characterization of peroxisomes, named for the fact that they produce hydrogen peroxide during their metabolic activity. They were found the way lysosomes were, by tracking an oddly behaving enzyme to its source. "Like mitochondria," says Dr. de Duve, "peroxisomes convert fuel to energy, but in an essentially wasteful manner. Their primitive form, mode of action, and broad distribution across species have led us to believe that they predate mitochondria. What we are seeing may be evolutionary descendants of a single ancestral particle that was present in the first eukaryotic cell (a cell with a true nucleus) from which all plants and animals are believed to have originated."

Paul Lazarow, who joined Dr. de Duve's Rockefeller laboratory as a graduate student in 1968, became fascinated by peroxisomes. "Although they are present in most and possibly all cell types and are very abundant in the liver," he says, "very little was known about their function or biogenesis; how their proteins are made and how the proteins are transported to and packaged in the organelle, a process that turns out to work differently in peroxisomes than in other organelles." His thesis research provided the answers to where in the liver cell the enzyme catalase is made and by what pathway it is conveyed to its peroxisomal abode.

After postdoctoral stints in Italy and at Stanford University, Dr. Lazarow returned to Rockefeller as a faculty member in 1975. "At that time," he says, "there were hints in the literature that peroxisomes might be involved in lipid (fat) metabolism; clofibrate and other drugs which are given to patients to lower blood lipid levels induced the proliferation of peroxisomes in experimental rats. We decided to investigate, and to our surprise found that peroxisomes were actually involved in fatty acid catabolism (metabolic breakdown)."

Concurrent with continuing studies of the biogenesis of peroxisomes, Dr. Lazarow is collaborating in an investigation of Zellweger's Syndrome, a very rare condition of infants born with no peroxisomes. "These children," he says, "live only a few months. They have abnormal lipid deposits and abnormal neural development. We're looking at what goes wrong when an organelle is missing. Obviously, this will give us information about what the organelle is doing when it's present."

Miklós Müller, from Hungary, was invited to join the laboratory when Dr. de Duve heard him report at a scientific meeting in London in 1963. "I had found lysosomes as the major digestive structure in protozoa," he says, "and in those days no one associated lysosomes with such primitive single-celled organisms. Dr. de Duve was interested in lysosomes in protozoa. I was interested in learning more biochemistry, and where better to do it than with de Duve. I thought I would stay only a year. Then we found peroxisomes in protozoa. A few years later, we found an organelle totally new to us, hydrogenosomes."

The discovery of hydrogenosomes resulted from a project begun with Donald Lindmark, another member of the group at the time, to find an experimental organism that would have peroxisomes but no mitochondria. "Through studies of electronmicroscopic images and our knowledge of comparative biochemistry," says Dr. Müller, "we came upon what we thought were likely candidates, a group of protozoa called trichomonads. They contained structures that looked like peroxisomes and their enzymes looked like they might be peroxisomal enzymes. But when I started my cell fractionation everything turned upside down. We found out that this organelle was making hydrogen, something done among animals, it turns out, by only trichomonads and one other small group of anaerobic (non oxygen-breathing) protozoa. So we named the organelles hydrogenosomes. What is their ancestry, their position in evolution we don't know, but we do know that among the trichomonads, *T. vaginalis* causes one of the most widespread sexually transmitted diseases in the United States.

"For a number of years there had been a drug on the market, metronidazole, that worked very well in the treatment of *T. vaginalis,* but its mode of action was unknown. We began to use the drug as a biochemical tool to understand how the organism works, which of course taught us a lot about how the drug works; its toxicity results from interaction with the enzymes in hydrogenosomes. At that time metronidazole was under attack as a possible carcinogen. Since it's the only effective drug for a

Electron microscopist Helen Shio. The instrument she works, one of a battery of different kinds of microscopes in the laboratory, is capable of a 200,000 fold magnification.

very prevalent and distressing problem, it was very useful to learn that its potential dangers were vastly exaggerated."

The search for understanding of cells remains the underlying basis of the laboratory's research. Just as the search has sometimes led into unexpected byways, it has also attracted scientists from seemingly unrelated areas. Carl Beyer's is immunology. When he started working in the lab, in 1974, immune cells were among many kinds of cells being examined structurally and biochemically. Although his work now focuses primarily on the components of the surface membranes of white cells that activate immune responses, he thinks of himself as a cell biologist as well as an immunologist. "Maybe I look like an odd thumb sticking up," he laughs, "but the way I feel, cells are cells." In another part of the lab, the cells Margaret Perkins studies are also rather specialized, red blood cells that have been invaded by malaria parasites.

"The common thread," Dr. de Duve explains, "is a common approach using the same multidisciplinary technology." To the methods of fractionation, microscopy, and biochemistry the laboratory has now added the new technologies of molecular biology, through which scientists seek to understand biological activity on the most intimate level, including what goes on in the place where those activities are programmed on the molecules of DNA: the nucleus. "The holy of holies," Dr. de Duve calls it.

SICK CELLS

In his new book, Dr. de Duve states: "Now that medical investigators are turning into subcellular and molecular detectives they are beginning to find out that a great many diseases are nothing but the manifestations of some digestive disturbance affecting certain cells...Dyspepsia, hyperacidity, constipation, and other digestive upsets are the common lot of mankind...Yet these troubles are nothing compared with the digestive ills that afflict cells."

The most dramatic examples of cellular "constipation" – lysosomal overloading – are caused by genetic deficiencies of some lysosomal enzymes. More than twenty-five such deficiencies have been found. Like Tay-Sachs disease, the best known, they often cause severe mental retardation and early death. Arteriosclerosis is also a form of cellular overloading, as are some kidney diseases. By contrast, when lysosomes unload where they shouldn't results can include rheumatoid arthritis, gout, and silicosis. In tuberculosis and leprosy, the lysosomes of immune cells fail to digest invading bacteria. All infections at base result from lysosomal failure.

In 1975 Dr. de Duve founded The International Institute of Cellular and Molecular Pathology, in Brussels, of which he is president. ICP is Dr. de Duve's "dream come true," his homage to his homeland and to Rockefeller, of which it is a replica on a smaller scale but with the same principles of untrammeled research in the service of human knowledge and health. One of the projects at ICP involves the designing of biological carriers to take drugs to lysosomes. "The idea," he says, "is to direct toxic chemicals to cells that one wishes to destroy – cancer cells, for example – by means of carrier substances that are taken up selectively by these cells. Knowledge of lysosomes has allowed the design of efficient targeted preparations of this kind that are now under clinical trial."

The medical applications that may derive from deeper insight into the intimate processes of cells are only beginning to be explored. The implications seem almost limitless. As Dr. de Duve comments: "The first great medical discoveries of the past century were made without the help of much understanding of cellular and molecular mechanisms. From the new findings of cellular and molecular biology will come, I am convinced, a second, even more remarkable era of discoveries. My generation has witnessed a revolution of knowledge that will be talked about a thousand years from now. I feel very privileged to have participated in it just a little."

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