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*Exploring cells with a Centrifuge*

The article, *Exploring cells with a Centrifuge*, by Christian de Duve, is about one of the most important discoveries in biomedicine in the second half of the 20th century. This discovery was made by using a centrifuge rather than a microscope to explore cells, and this method started a decade after the electron micrograph was used. (Before only microscopes were used to explore cells which made it very difficult to fully examine the cells more in depth and individually.) Scientists were also able to better examine and look at each cell's sub-cellular organelles. The experiment illustrated a mind-set for doing science that was not only integrated but innovative as well. For no other scientist had ever thought to look at cells in this way before. And, this new way of looking at cells helped scientists to learn more about each individual structure of an organelle by separating and isolating them from each other.

This experiment began in 1949 when Christian de Duve was following Albert Claude in his new electron microscopy techniques. However, the technique that Christian de Duve wanted to use was centrifugal fractionation because it was a method that was solid and reliable. And at the time, scientists Claude, Hogeboom, Schneider, and Palade had already described centrifugal fractionation in detail. For the fractions and the original homogenate could then be analyzed for their chemical composition and enzyme content. These details of the scientist's experiment were readily available for reference and all that was really needed was the necessary equipment to execute the experiment properly.

At first, all Christian de Duve and his team of scientists wanted to know was the localization of the enzyme glucose 6- phosphatase, which they thought might have a possible clue to the mechanism of action or lack of action of insulin on the liver cell. However, fortunately for Christian de Duve, this is not how the experiment unfolded. Christian de Duve thought that centrifugally isolated fractions would be impure, thinking that the cell organelles might be difficult to resolve quantitatively. Aware of the limitations of the light microscopic examinations of the fractions, Christian de Duve tried to extend the biochemical interpretation as far as possible. What Christian de Duve and his team did was look at each individual enzyme, contemplating its distribution between all the fractions instead of looking at each fraction separately and focusing on its enzyme content.

It was believed at first that sub-cellular particles might be inseparable from each other due to their overlapping of size and their densities. However, it was possible for them to obtain pure samples still by cutting over the non-overlapping parts of the populations, yet this method also was on the fringe of biased sampling. But, almost complete separation was almost achieved in 1962 by Baudhuin, Wibo, and Wattiaux when these scientists discovered that the pretreatment of the animals with Triton WR-1339 had a great decrease in their density of lysosomes. (Which was due to the accumulation of the Triton within these particles.) From uncovering these large scale separations of the three populations it further allowed a variety of biochemical studies to be available, that were not available before the pretreatment of animals with Triton WR-1339. Evidence that proved useful for Christian de Duve was based off of enzyme latency.

Due to knowing that the impermeability of particle membranes to one or more of the substrates used in the assay of enzymes, many particle bound enzymes failed to display activity in vitro as long as the membrane surrounding them was intact. If two or more enzymes are

present together in the same particle, they would be released together in this type of experiment and if they were different particles, they would come out separately. Through this,

Christian de Duve found that there was more activity of enzymes after fractionization than before. Through marker assays, Christian de Duve was able to see where particles go.

A major problem that existed for Christian de Duve was that the available techniques did not measure up to the kind of information that he was hoping to get from his experiment. The answer to his problem came in the form of density gradient centrifugation. This new technique offered a resolution because it used density as well as a sedimentation coefficient as a separation parameter. The result of Christian de Duve's experiments confirmed and extended the earlier findings that there was a distinct existence of three distinct groups of enzymes defined by their centrifugal behavior. By fitting these results to a theoretical equation, Christian de Duve and his team were able to evaluate a number of physical parameters for each putative particle population.

Although the results were analytically satisfactory, the results of his experiment fell short from the actual proof. Since they had unfortunately confirmed that distinct populations of sub cellular particles might prove intrinsically inseparable quantitatively due to overlapping of size and density distributions. The advantage of Christian de Duve's analytical approach is that it is widely applicable and it can provide a considerable amount of quantitative information even with a relatively poor resolving power. The key importance to this kind of methodology is that information is derived not from the properties of specific fractions believed to approximate a given intracellular component but rather in a manner in which properties are distributed over a large number of fractions, which together represent the whole tissue. Christian de Duve did not realize how much his new findings could be used in the medical field until well after his findings

occurred. For instance the possibility that lysosomes might accidentally become ruptured under certain circumstances and kill or injure their host cells as a result was considered right after he got his first clues to the existence of these particles. Christian de Duve's findings don't only enrich people's knowledge of exploring cells but also can be used in concurring disease. What this particular experiment illustrated was a mind-set for doing science that was not only integrated but innovative as well. For it opened opportunities to learn and understand more about each individual structure of an organelle by separating and isolating them from each other.