A considerable proportion of the activities of several hydrolases was found to be sedimentable in cell-free extracts from root tips of corn seedlings. Mitochondrial fractions were subjected to density gradient centrifugation: lysosomes differing in density and enzyme contents were isolated and identified with small vacuoles. These structures were interpreted as early stages of the development of large vacuoles typical for mature plant cells. [The SCI® indicates that this paper has been cited over 120 times since 1968.]

Philippe Matile
Department of General Botany
Swiss Federal Institute of Technology
8092 Zürich
Switzerland

November 17, 1981

"Digestive enzymes began to incite my curiosity when I looked up the trivial name Neurospora crassa and began to figure out how a mold eats bread. I came to the conclusion that the secretion of hydrolases, such as amylase and protease, in conjunction with the absorption of low molecular weight products of extracellular digestion would be the most likely strategy of a bread mold. This was in 1962, and I was a research fellow at the Rockefeller Institute at that time. I decided to study the subcellular compartmentation and secretion of proteases in Neurospora crassa and discovered that intracellular proteases are located in vesicles which are now known to be identical with small vacuoles.

"It was during my stay in New York that I first encountered the term lysosome. In fact, the early review which de Duve wrote for Scientific American was an enlightenment. Since it was evident that intracellular digestive processes must also occur in plants, for instance in senescing leaves when the protein is mobilized, I was convinced that lysosomes must also exist in plant cells."

"After my return to Zürich I started the search for plant lysosomes. It was initially rather disappointing. In conventionally prepared homogenates from plant tissues, the bulk of lysosomal marker enzymes such as acid phosphatase and protease turned out to be soluble. Apparently, other researchers came to the same conclusion. In fact, it was easy to prove the nonexistence of plant lysosomes by simply subjecting a cell-free extract to the standard fractionation procedure developed for rat liver by de Duve's group. "Plant cells possess rigid walls and rather drastic means are necessary for the mechanical disintegration of tissues. This trivial experience led to the idea that plant lysosomes may be so fragile that they are inevitably disrupted by tissue homogenization; the central vacuole is such a delicate organelle. Yet, it appeared impossible at that time to isolate them and check their lysosomal properties. Therefore, I selected root tips hoping that I would eventually be able to isolate the small vacuoles of meristematic cells. A technique based on the slicing of plasmolysed corn root tips indeed allowed the liberation and subsequent isolation of small vacuoles containing several hydrolase activities. Andres Wiemken, a graduate student at that time, succeeded in the isolation of vacuoles from yeast protoplasts whose lysosomal properties further supported the hypothesis that the vacuole is the analogous plant organelle to the animal lysosome.

"Since the slicing technique was tedious and only gave very small yields of vacuoles, I looked for alternative procedures. I noticed that upon careful grinding of root tips in the presence of sand and a suitable medium, considerable proportions of various hydrolases are sedimentable. It was relatively easy to develop a technique for subfractionating a mitochondrial preparation and to identify the fractions containing hydrolase as small vacuoles. The above cited paper has a second part coauthored with Hans Moor in which the morphological observations on meristematic vacuoles, their origin, and dynamism as they appear from the freeze-etchings are summarized."

"The paper which now has been declared a classic is, in my personal judgment, not my best paper, yet it has been cited frequently because it is among the first publications dealing with plant lysosomes. Although the lysosomal properties of mature vacuoles have now been established, it still remains to be explained how the plant cell uses its lysotic compartment."