possible that β -glycerophosphatase has a predominantly digestive role, whether auto- or heterophagic, and that the naphthol phosphatase described in this paper has a completely different role in cell metabolism.

The distribution of the naphthol phosphatase in both lysosomes and endoplasmic reticulum is similar to that of a number of other acid hydrolases, e.g. esterases (Holt & Hicks, 1966), β -glucuronidase (Fishman, Goldman & De Lellis, 1967) and arylsulphatase (Dodgson, Rose & Spencer, 1957; Dodgson, Spencer & Thomas, 1954, 1955; Viala & Gianetto, 1955; Milsom, Rose & Dodgson, 1968).

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Vacuoles as Lysosomes of Plant Cells

By PH. MATILE. (*Department of General Botany, Swiss Federal Institute of Technology, Zurich, Switzerland*)

The investigation of plant subcellular particles has led to the suggestion that lysosome-like structures as defined by de Duve are absent from plant cells. If plant tissues are homogenized by using conventional techniques the lysosomal enzymes are largely present in the soluble cell fraction. Nevertheless, with appropriate methods it can be shown that these enzymes are localized in cell compartments corresponding to the lysosomes of animal cells.

In yeast cells they are identical with the vacuoles that are destroyed on conventional homogenization. The isolation of intact vacuoles has been achieved by using naked protoplasts instead of whole cells. Under suitable conditions the lysis of osmotically labilized protoplasts is accompanied by the release of vacuoles, which can be separated by centrifugation in the presence of Ficoll. Fractions of isolated yeast vacuoles contain very high specific activities of the lysosomal enzymes. The analysis of various cell fractions suggests that the vacuoles are the only lysosomes of the yeast cell (Matile & Wiemken, 1967).

Evidence favouring the classification of higher plant vacuoles as lysosomes comes from several lines. On cytochemical grounds the localization of acid phosphatase in meristematic vacuoles of wheat embryos (Poux, 1963a), autophagic vacuoles of starving *Euglena* (Brandes & Bertini, 1964) and aleurone vacuoles (Poux, 1963b, 1965) has been established. However, acid phosphatase seems to represent a doubtful marker enzyme for lysosomal structures. It may be completely absent from lysosomes as it is the case in yeast cells. In addition, its exact function in intracellular digestion has not yet been elucidated, and, finally, in higher-plant cells several acid phosphatases differing with regard to intracellular distribution are present (Ph. Matile, unpublished work). Therefore, the evidence based on isolated organelles seems to be more conclusive.

In meristematic vacuoles isolated from root-tip cells of corn seedlings a dozen acid hydrolases including proteolytic enzymes, nucleases, glucosidases, esterases and phosphatase are localized (Matile, 1966, 1968a; Ph. Matile, unpublished work). It is noteworthy that the largest vacuoles that can be extracted from root-tip cells are not sedimentable in the presence of comparatively low concentrations of sucrose. Thus these lysosomes cannot be prepared if sucrose solutions are used as media for homogenization and centrifugation.

According to the view of classical cytologists the

large vacuoles of differentiated cells develop from small meristematic vacuoles. This kind of ontogeny has been confirmed by means of electron microscopy of frozen-etched root tips. Moreover, evidence favouring the origin of the small vacuoles from the endoplasmic reticulum has been obtained (Matile & Moor, 1968). The direct analysis of isolated central vacuoles has not yet been possible because of experimental difficulties. Therefore, the classification of these structures as lysosomes of parenchymatous cells is based on indirect evidence only.

A special type of small vacuole with lysosomal properties has been isolated from the latex of *Hevea brasiliensis* by Pujarnicle (1968). Finally, in aleurone vacuoles isolated from cotyledons of dry cotton seeds (Yatsu & Jacks, 1968) and germinating pea seeds (Matile, 1968b) lysosomal enzymes are localized.

Hence with respect to their lysosomal function the morphologically diverse vacuoles of higher plant cells seem to represent modifications of a universal organelle.

The role of plant lysosomes in intracellular digestion has been studied both morphologically and biochemically. In yeast, the total activities of lysosomal enzymes depend on the growth phase: they are low during exponential-growth periods of *Saccharomyces* on glucose medium, and tend to increase markedly during induction periods, when cells adapt either to respiratory or to fermentation metabolism. In addition, stationary-phase cells contain high activities of lysosomal enzymes (A. Wiemken, unpublished work). It appears from these results that the activities of acid hydrolases reflect the rate of cellular lytic processes (turnover), which is low in exponentially growing cells and high in adapting or stationary-phase cells.

A similar correspondence between the activities of lysosomal enzymes and lytic events has been observed in senescing petals of *Ipomoea purpurea*. The mobilization of the cytoplasm in wilting flowers is accompanied by the formation of new lysosomal enzymes such as protease, ribonuclease and β -glucosidase. Simultaneously, the total protein and RNA content of the petals decreases rapidly. Individual lytic enzymes are formed at different stages of senescence (F. Winklenbach, unpublished work). It appears from the study of such an extreme case of differentiation, which ends with cell death, that the compartmentation of lytic and synthetic processes is maintained until the mobilization of the cytoplasm is almost entirely completed. Therefore it must be assumed that the tonoplast remains continuous, the lysosomal enzymes not being released into the cytoplasm. In fact, the morphological investigation of various processes of differentiation has proved the validity of this assumption. In detail, studies have been

carried out of senescence of petals, mobilization of cytoplasm in fruiting bodies of *Coprinus lagopus*, mobilization in storage cells of seeds, differentiation in root tips and adaptation in yeast cells. All of the processes studied were characterized by the continuity of the tonoplast, i.e. by the strict compartmentation of the lysosomal enzymes. The intracellular digestion of cytoplasmic material is initiated by pinocytosis-like activities of the tonoplasts resulting in the formation of intravacuolar vesicles. These structures are unstable and release their cytoplasmic content into the cell sap, where it is degraded by the action of the lysosomal enzymes. The release of lysosomal enzymes into the cytoplasm must be regarded as a consequence of cell death rather than an event indicating a vital process such as differentiation.

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Subcellular Localization and Function of Hydrolases in Differentiating Plant Cells

by P. B. GAHAN. (*Woolwich Polytechnic, London, S.E. 18*)

Initial biochemical studies on plant materials failed to reveal structures identifiable as lysosomes according to the definition of de Duve (1959), owing apparently to the mode of tissue preparation employed to break open the plant cells (Gahan, 1968; Matile, 1969).

In order to avoid the deleterious tissue-preparation procedures, other workers adopted a cytochemical approach to the study of the distribution of acid hydrolases. Thus, a number of workers reported the presence of various acid hydrolases at particulate sites in the cytoplasm (Jensen, 1956; Avers, 1961; Walek-Czernecka, 1962; Gahan, 1965; Gorska-Brylase, 1965). In studies of acid phosphatase, activation of the 'particulate sites' was achieved by freezing and thawing the sections or by pre-incubation of the sections at 37° in acetate buffer, pH 5, suggesting a lysosomal behaviour (Gahan, 1965). Cells of crown gall grown in culture were treated to a double reaction while under continuous microscopic observation, showing that