whereas the azo-dye method produces a strictly lysosomal localization of acid phosphatase. Enzyme distribution demonstrated by the method of Holt & Hicks (1961) closely resembles that obtained by the azo-dye method, except for occasional cytoplasmic staining. The use of frozen sections, however, results in some loss of ultrastructure.

Combined electron-cytochemical and ultrastructural studies permit a tentative classification, based on that of de Duve & Wattiaux (1966), of the vacuole population of the epithelial cells. The fate of the primary lysosomes, which appear to arise from Golgi vacuoles, falls into three categories: (1) secretion into the gut lumen, where it is likely that the lysosomal enzymes are released for digestive purposes, (2) fusion with each other to form larger vacuoles and (3) direct fusion with secondary lysosomes. Primary lysosomes are also concerned with the pinching off of small portions of cytoplasm during apocrine secretion.

There is no evidence of heterophagy in the digestive epithelial cells and many of the secondary lysosomes are obviously autophagic in origin, containing remains of mitochondria and endoplasmic reticulum. The autophagic secondary lysosomes or autolysosomes contain acid phosphatase at all stages of development and no autophagosomes as such have been observed. It is possible to distinguish between early and mature autolysosomes. The early forms in general contain easily recognizable cytoplasmic organelles since lysis at this stage is minimal. Such vacuoles are usually surrounded by two membranes that are presumably derived from the sequestration membranes, whereas the mature autolysosomes are invariably surrounded by a single membrane, as are the telolysosomes and residual bodies.

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Lysosomal and Extralysosomal Localization of Acid Phosphatases in Rat Sensory Ganglia

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Classification of sensory ganglia into small 'dark' and large 'light' neurones is well known (Crosby, Humphrey & Lauer, 1962).

Demonstration of acid phosphatase activity in rat trigeminal and spinal ganglion cells, with

β -glycerophosphate as a substrate (Gomori, 1952), showed a particulate-granular localization of the reaction product in the small cells. Activity in the large neurones appeared as a network of fine filaments (Kalina & Bubis, 1968). The β -glycerophosphatase reaction in the large neurones was sensitive to aldehydes but was activated by 0.1 mM-molybdate; the reaction in the small cells was not affected. Activity of acid phosphatase when demonstrated with naphthol AS/B1 phosphate as a substrate (Burstone, 1958) showed a granular type of reaction in both types of cells. Similar results were also obtained in mouse, rabbit and human preparations of sensory ganglia.

Observation under the electron microscope showed the localization of β -glycerophosphatase to be only in lysosomes. In the large cells the reaction product appeared in the cisternae and vesicles of the Golgi apparatus and also attached to the outer surface of the endoplasmic-reticulum membranes.

The results suggested the possibility that more than one acid phosphatase was present in the small and the large neurones and that the enzymes were differently localized within the cells. This possibility was tested by a chromatographic study on acrylamide gels. A homogenate of trigeminal or spinal ganglia was separated on the gel into seven to nine bands of β -glycerophosphatase, but only three of them showed the naphthol AS/B1 phosphatase activity. Gel electrophoresis of Triton-treated cytoplasmic fractions obtained by differential centrifugation showed that the lysosomal fraction contained four bands that exhibited β -glycerophosphatase activity, two of which had naphthol AS/B1 phosphatase activity. The microsomal fraction contained three β -glycerophosphatase-reacting bands and only a trace of naphthol AS/B1 phosphatase activity. Without Triton treatment no migration occurred.

These results indicate the existence of multiple forms, or isoenzymes, of acid phosphatase in the sensory ganglia of rat and other species. These forms are probably differently localized in the small and the large neurones. It is possible that the different localization, although its meaning is unclear, is of biological significance and may suggest that the two groups of cells differ in function.

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