FUNCTIONAL ANALOGY OF PLANT VACUOLES WITH ANIMAL LYSOSONES

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After the discovery of lysosomes in animal cells by de Duve in early fifties, there was a controversy about the existence of lysosomes in plant cells. While Berjak observed lysosome-like organelle in the root cap of Zea mays employing an electron microscope, Matile suggested that the vacuoles represent lysosomes in plant cells. This was later confirmed by the presence of characteristic hydrolases and latency of these enzymes in the vacuoles. Also, these criteria are used to detect lysosomes in animal cells which establish the structural analogy of plant vacuoles with animal lysosomes. The present investigation was undertaken to verify the functional analogy of plant vacuoles with animal lysosomes by feeding acidic orange (AO), a foreign substance, to the plant cells and observing the effects on vacuoles.

The secondary roots of Vicia faba, while attached to seedlings, were treated with 20 and 50 ppm aqueous AO solution for periods ranging from 6 hr to 7 days. The root tips were then cut and processed for electron microscopy.

Ultrathin sections of the control root tip cells reveal typical ultrastructure (figure 1) with nucleolus, nucleus, nuclear membrane, profiles of endoplasmic reticulum, mitochondria, Golgi bodies and amyloplast with electron-transparent starch grains. The vacuoles are generally empty. Presence of prometaphase chromosomes shows that the cells actively divide. In the electron micrograph of cells treated with 20 ppm AO for 6 hr (figure 2) all the cell organelles appear to be normal except the prominent invaginations of the tonoplast (single arrow). In addition, vacuoles contain intravacuolar bodies ranging from 0.2 to 2 μ in diameter. These bodies appear to be of protoplasmic origin owing to their similarity in structure and density with that of protoplasm. As treatment prolonged to 12 hr the amount of intravacuolar bodies increased (figure 3). Some of these bodies contain cytoplasmic organelles like mitochondria (single arrow) and presumably partially digested thin protoplasmic strands (double arrow). The cells, however, appear to be healthy as evident from the presence of metaphase chromosomes which reflects the continuity of cell division. When the cells were treated for longer periods (7 days) the intravacuolar bodies were more complicated and varied (figure 4). The vacuoles displayed numerous intravacuolar bodies ranging from 0.3 to 3 μ in diameter; short protoplasmic strands were also distinct. The cells were, however, still dividing. With the higher concentration (50 ppm) of AO when the cells were treated for 48 hr (figure 5) two types of intravacuolar bodies were discernible (i) similar in structure and density to protoplasm (single arrow) (ii) denser than the protoplasm and appear to be granular abiological mass (double arrow).

AO is a cationic substance and is known to interact with many anionic substances like nucleic acids, proteins, lipoproteins and phospholipids. Some of these substrates are available in the membranes, and as a result AO preferentially binds to membranous structures in the cell. Such attachment of AO leads to general toxicity which is evident from the report that AO inhibits RNA synthesis in vivo and is also known to cause structural abnormality in mitochondria and plastids of wheat root meristem. It is possible that in the present system mitochondria and plastids become defective due to excessive binding of the dye and as soon as these protoplasmic regions come in contact with tonoplast, they are recognized and an invagination appears in tonoplast which ultimately engulfs the defective organelles along with surrounding protoplasm. This process is known as autophagy. Numerous cases of autophagy have been reported in the literature. The literature on animal cells is rich with reports of autophagy induced by a variety of chemicals like glucagon, actinomycin-D, puromycin and cycloheximide.

It is generally agreed that cytoplasmic organelles, once enclosed inside vacuoles, are attacked by vacuolar hydrolytic enzymes and subsequently degraded. Likewise in the present study mitochondria can be
Figures 1–5. Electron micrographs of control and acridine orange (AO) treated root tip cells of *Vicia faba*. Fixed in 1.5% glutaraldehyde-formaldehyde followed by post fixation in 2% osmium tetroxide and staining with uranyl acetate and lead citrate. 1. Control cells with normal nucleus (N), prometaphase chromosome (CHR), nucleolus (Nu), mitochondria (M), amylloplast (A) and distinct empty vacuoles (V). ×3,000. 2. 20 ppm AO for 6 hr treated cells showing prominent invagination in the tonoplast (single arrow). Intravacuolar bodies (IVBs), ranging from 0.2 to 2 μ, are distinct in the vacuoles. ×3,000. 3. Prolonged period of treatment (12 hr) leads to increased amount of IVBs some of which contain mitochondria (single arrow). Partially digested proplasmic strands are also visible (double arrows). ×6,250. 4. More complicated and varied IVBs (0.3–3 μ in diameter) in the cells treated with 20 ppm for 7 days. ×4,500. 5. Two types of IVBs, (i) similar in structure and density with proplasm (single arrow) and (ii) granular abiological mass denser than proplasm (double arrow), are distinct in the cells treated with 50 ppm AO for 48 hr. ×15,000.

seen in various stages of digestion. The electron-dense intravacuolar abiological material is presumably dye particle groups which are taken into the vacuoles after the available reaction sites in the cell are saturated. Such a process in which foreign material is taken into vacuoles or lysosomes, is known as heterophagy. The uptake of neutral red, similar in structure and molecular weight, has been shown in the secondary vacuoles of *Tradescantia* hair cells and lysosome-like particles (vacuoles) of potato leaf epidermal cells. The accumulation of AO in the vacuoles of fungal spores (*Ceratocystis ulmi, Botrytis cinerea* and *Cryptococcus neoformans*) supports our contention that electron-dense material is really acridine orange complexed with heavy metals like osmium tetroxide and/or uranyl acetate.

The structural analogy of plant vacuoles with animal lysosomes has already been demonstrated by the presence of two or more characteristic hydrolases within membrane-bound bodies and the latency of enzymes (*loc. cit.*). The plant vacuoles, in the present study, have been shown to perform the function of animal lysosomes. This establishes the functional analogy of plant vacuoles with animal lysosomes. In addition, since these vacuoles have been demonstrated to be of autophagic as well as heterophagic in
NUCLEAR POLYHEDROSIS OF SESAMIA INFERENS (NOCTUIDAE: LEPIDOPTERA)
THE PINK STEM BORER OF RICE

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SESAMIA inferens Walker is a polyphagous insect pest infesting sugarcane, sorghum, finger millet and rice. During the course of laboratory rearing, larvae of S. inferens were consistently found dead in the insectary, due to polyhedrosis. Microscopic examination of the haemolymph and tissue smears revealed a large number of refractile polyhedral bodies which were negative to staining by Giemsa. Rice stem pieces dipped in partially purified polyhedral suspension, when fed to healthy laboratory reared test larvae, caused the infection. The infected larvae came out of the stems. As the disease intensified, the ventral side of the infected larvae turned whitish, starting from fifth segment of the body and gradually progressing towards the end. Whitening was more prominent at the intersegmental membrane. The cadavers were dull white to light brown in colour. Larvae were seen either sticking to the inner surface of rearing jar or hanging by prolegs from its top. The integument was very fragile and easily ruptured, liberating the liquefied body contents. The test larvae died in 3–5 days.

The dead larvae decomposed in distilled water for one month was centrifuged by alternate low (1000 g) and high (5000 g) speed centrifugation for 10 and 20 min. respectively. Highly purified polyhedral suspension was obtained by sucrose 20 to 60% (w/v) density gradient. These polyhedra were placed on the ‘Formvar’-coated grids and observed under Hitachi electron microscope with 50 kV operating voltage. The polyhedra were irregular in shape with an average size of 0.84 μm (figure 1). In order to locate the virions in the polyhedra, crystallized protein of polyhedra was selectively disaggregated by treatment with thioglycolate at pH 10 for 1 min. Dissolution was performed on the specimen holder (‘Formvar’-coated grid). These preparations were stained with 3% uranyl acetate. Thioglycolate acted upon the polyhedral protein and dissolved the polyhedra liberating the virions from the polyhedral inclusions (figure 2). The virions were rod-shaped and measured 215×35 nm. They were enveloped in bundles of 2 or 3 (figure 2). Two or more virions in a bundle were reported from nuclear polyhedroses of Euprostis similis and Bellura gosyntoides. After shedding of virus particles, the envelopes appeared spherical. The bundles appeared to be randomly distributed in the polyhedra.


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