The Cytochemistry of Some Enzyme Activities in *Stellaria media* Embryos

Several enzymes are known to be important to the growth and differentiation of cells in both plants and animals[1-3]. Among the enzymes of consequence in plants are alkaline phosphatase[4], acid phosphatase[5,6], and cytochrome oxidase[7]. We have shown that these enzymes are also important during the early stages of tissue differentiation in embryos of *Stellaria media* (L.) Cyrill. The developmental morphology of *S. media* has been documented[8] and its topological cytochemistry has been studied[9].

Materials and methods. All tests were performed on freshly dissected heart-shaped embryos of *S. media* 5–6 days after fertilization. Alkaline phosphatase was demonstrated using the Gomori cobalt sulfide method[10], acid phosphatase using the lead sulfide procedure[5], and cytochrome oxidase with the Nadi reaction[11].

Controls for the alkaline and acid phosphatase localizations consisted of embryos of similar sizes and developmental stages incubated in reaction media lacking the appropriate substrate (sodium glycerophosphate in the case of acid phosphatase and adenosine-3-phosphate in the case of alkaline phosphatase). Controls for cytochrome oxidase were embryos of similar size and development incubated in a complete reaction medium plus 0.005*M* sodium azide. All controls were negative.

Results and discussion. Acid phosphatase activity in the suspensor was minimal when compared with the embryo proper (Figure 1). Within the embryo most of the enzyme activity occurred in the protoderm cells. However, those cells which form the embryonic root cap, the 'hypophysis', showed no acid phosphatase activity. It appeared that this enzyme was active in only those protoderm cells which differentiate into adult epidermal tissues but not in those which form the root cap. Chemically this enzyme has been shown to mediate the transfer of phosphate esters, and it has been demonstrated in a wide variety of differentiating tissues including regenerating urodele limbs[3] and *Phleum* root meristems[12]. AVERS has found it to be associated with the mitochondrial, ribosomal and Golgi fractions of the meristem cells.

Alkaline phosphatase and cytochrome oxidase were active throughout the developing embryo and its suspensor. Alkaline phosphatase was apparent in the 3 primary meristematic tissues (protoderm, ground meristem and procambium) as well as the cytoplasmic proteinoplasts of the suspensor cells (Figure 2). Cytochrome oxidase was similar in its distribution (Figure 3) except in the suspensor cell cytoplasm where it was not associated with the proteinoplasts (compare inserts in Figures 2 and 3). The restriction of alkaline phosphatase to the proteinoplasts corresponded with the presence of rich lipoprotein deposits in these structures[9]. It evidently was involved with the hydrolysis and transfer of phosphate ester bonds associated with the lipoprotein complexes. This would help to explain the formation of the proteinoplasts during the first 5 days after fertilization and their subsequent immolation during cotyledon formation.

Cytochrome oxidase is one of the enzymes associated with the cytochrome electron transport system which is so important to cellular respiration and the generation of ATP. As such, its presence was a sure indicator of mitochondria and a high rate of metabolic activity.

The function of the suspensor has been in dispute since E. C. JEFFREY suggested in the late 19th Century that it merely pushed the embryo deeper into the prothallial tissue. This idea has been cited as recently as 1950 by MAHESHWARI[13] who stated, '...in the majority of angiosperms the suspensor has no special function except that of pushing the embryo into the endosperm, where it is surrounded by cells containing abundant food mate-

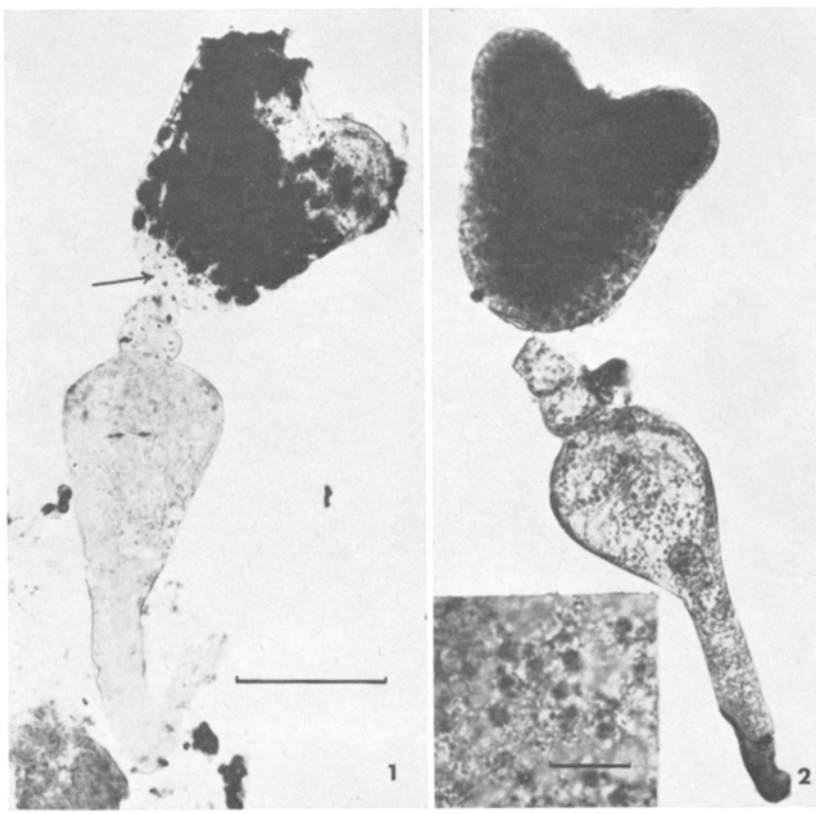

Fig. 1. Embryo with attached suspensor, 5–6 days after fertilization, treated to show acid phosphatase activity. Note the dark staining cells of the protoderm layer and the lack of enzyme activity in the suspensor, the interior cells of the embryo, and the 'hypophysis' (arrow). Line indicates 100 μ.

Fig. 2. Same stage embryo illustrating alkaline phosphatase activity. Note the concentration of the enzyme uniformly throughout the embryonic cells but only in the proteinoplasts of the suspensor. Insert is an enlargement of the basal suspensor cell cytoplasm showing enzyme activity associated with the proteinoplasts. Line indicates 10 μ.

rials'. WARDLAW[14] does not agree with a purely mechanical function for the suspensor, and suggests that it has a physiological role in embryogeny. HACCIUS'[15] work with tissue culture embryos supports the idea that it has a physiological role in the formation of the growing embryo. The evidence from topological histochemical studies on *S. media*[9] and from the current enzyme studies also supports WARDLAW's premise that the suspensor has a biochemical-physiological function.

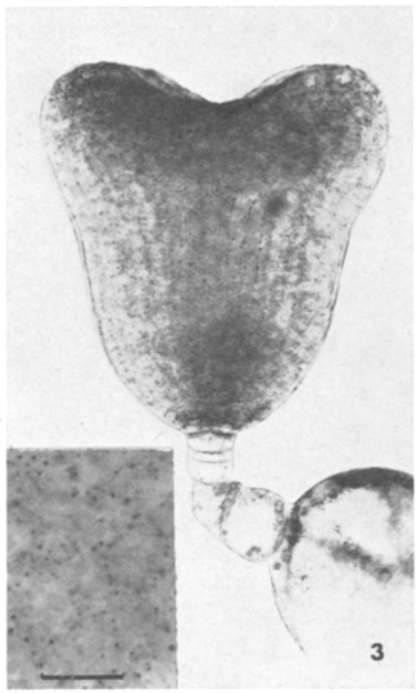

Fig. 3. 5–6-day-old embryo treated to illustrate cytochrome oxidase activity. Note the presence of activity in all the embryonic cells with concentrations in the developing regions of the cotyledons, the root meristem and the procambial strands. Some activity can also be seen in the suspensor. Insert shows an enlargement of the basal suspensor cell cytoplasm in which the cytochrome oxidase activity is seen not to be associated with the proteinoplasts, but instead is found in the matrix around these plastids. Line indicates 10 μ.

Zusammenfassung. Zytochemischer Nachweis der alkalischen und und sauren Phosphatase, sowie der zytochromischen Oxydase in den herzförmigen Embryonen der Dikotyledon *Stellaria media*. Die Fermente erwiesen sich als wichtig für normales Wachstum und die Differenzierung der Embryonen.

H. N. PRITCHARD and K. A. BERGSTRESSER[16]

Department of Biology, Lehigh University, Bethlehem (Pennsylvania 18015, USA), 12 May 1969.

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Influence of *Curvularia* Infection of the Free and Bound Amino Acid Composition of Orange (*Citrus aurantium* L.) Fruits

Curvularia lunata (Wakker) Boedijn is a very important pathogen of orange (*Citrus aurantium* Christm.) fruits. The fungus causes severe rotting of oranges during the post-harvest phase. No attempt has so far been made to study the changes in the free and bound amino acid composition of the orange fruits brought about by the infection of the fungus. Considering it as an important contribution to our present knowledge of this aspect, an attempt was made to investigate it.

Just ripe fruits of same age were inoculated with *Curvularia lunata* and were incubated at 25 ± 1 °C for 15 days. Extracts of 1 g each of healthy and diseased tissues were prepared separately with 25 ml of 80% ethanol. They were filtered and evaporated to dryness. The residues left after evaporation were dissolved each in 1 ml of 20% ethanol and were centrifuged at 2000 rpm for 30 min. The clear supernatant liquid was decanted and used for analysis of free amino acids.

In order to release the bound amino acids, the alcohol extracted residues left on the filter papers and the colloidal protein settled in the centrifuge tubes were combined and hydrolyzed with the help of 6*N* HCl at 15 lb. pressure for 30 min. A pinch of stanous chloride ($SnCl_2$) was added to avoid humin formation. The hydrolyzed residues were filtered through buchner funnels and the hydrolysates were adjusted to 1 ml in each case. They were subsequently centrifuged and used for the analysis of the bound amino acids.

For complete resolution of diverse amino acids, two-dimensional ascending chromatographic technique described by CONSDON et al.[1] was followed. Adjusted concentrations of the soluble and insoluble fractions of different types of tissues were spotted on Whatman No. 1 filter paper (28 × 28 cm). PARTRIDGE's[2] solvent, as modified by FOWDEN[3], i.e. phenol-ammonia-water (80:3:20, V/V) was used as the first running solvent and *n*-butanol-acetic acid-water (4:1:5, V/V) as the second one. The chromatograms were sprayed with 0.1% mixture of ninhydrin (indane-trione hydrate) in *n*-butanol (W/V). They

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