50% required 0.1 mM-iodine when the enzyme was 8.15 nM. The reaction was complete in a few seconds. Mercaptoethanol and mercaptoethylamine reactivate the partially inhibited enzyme. However, neither 0.1 M-cysteine nor 0.1 M-ascorbate reactivate the enzyme.

The variation of $V_{\max}$ of native invertase with pH shows clearly that a single protonated acid with a pK_a of about 6.8 is necessary for catalysis. The $V_{\max}$ of iodine-treated invertase shows the same pH-dependence; the pK_a in the derivative is also about 6.8. The actual values of $V_{\max}$ for native invertase are 1.5–2 times the values for iodine-treated invertase. Clearly, the acid group with a pK_a of 6.8 is not chemically modified by the iodine treatment. The group is presumed from the pK_a value to be an imidazole.

The kinetics of the reactivation reveal that the rate is first-order both in enzyme and in mercaptoethanol. A plot of the logarithm of the second-order rate constant against pH shows two straight lines of slope 0 and +1, which intersect at pH 6.2.

Spectral evidence shows that iodotyrosine is not formed. In addition, nitration of tyrosine with tetranitromethane does not affect enzyme activity.

We conclude that an imidazolium cation with a pK_a of 6.8 participates in catalysis but that the iodine does not react with this group. Since the iodine inhibition is reversible, the oxidation of cysteine to cystine is a probable explanation. The inability of cysteine to reactivate is probably due to charge repulsion effected by the presence of a carboxylate anion at or near the active centre. The kinetics of reactivation suggest that an acid group with a pK_a of 6.2 is involved in its base form. This group may catalytically enhance the rate of reduction or it may be involved in a pH-dependent conformational equilibrium. The decrease in $V_{\max}$ after iodine treatment may be caused by cross-linking of sub-units or molecules, the number of functioning active sites thus being decreased.

Euler, H. von & Landergren, S. (1922). *Biochem. Z.* **131**, 386.

Myrbäck, K. & Willstaedt, E. (1957). *Ark. Kemi*, **13**, 179.

Waheed, A. & Shall, S. (1967). *Abstr. 4th Meet. Fed. Europ. biochem. Soc., Oslo*, no. 481.

Hydrolase Activities in Differentiating Plant Tissues

By JEAN McLEAN (*Queen Elizabeth College, London, W. 8*) and P. B. GAHAN (*Woolwich Polytechnic, London, S.E. 18*)

The distribution of acid phosphatases in differentiating plant tissues has been described (Gahan &

McLean, 1967). In the present study the distribution of esterases was determined and compared with that of the phosphatases at the tissue, cell and subcellular levels. The hydrolases were examined in unfixed, frozen sections of tissues from *Vicia faba* and *Lycopersicon esculentum* (Gahan, McLean, Kalina & Sharma, 1967) by incubation in the reaction media at 37° for times of 2–60 min. Esterases were assayed with naphthol AS/D acetate as substrate (Gahan & McLean, 1967; Livingstone, Coombs, Franks, Gahan & Maggi, 1969), and phosphatases with either naphthol AS/B1 phosphate (Gahan & McLean, 1967) or β -glycerophosphate (Holt, 1959; Bitensky, 1962) as substrates.

Esterase and phosphatase activities were similar in mature tissues. In contrast, most variable activity was seen in the differentiating tissues. Thus, whereas phosphatase activity was high in apical initials and pro-cortical tissues, esterase activity was very low. Conversely, pro-cambial tissues revealed high esterase and low phosphatase activities. The exception to this was the primary phloem which showed high esterase and phosphatase activity.

In cells that contained both esterases and phosphatases, the two groups of hydrolases were demonstrable in the same particles, which tended to occupy a more central position in the cytoplasm of the cell. Cells that exhibited elongation and expansion of the wall contained a second group of particles, which were adpressed to the wall. These particles showed no detectable phosphatase activity but considerable esterase activity.

High-resolution cytochemical studies have shown some of the particles containing the esterase end-product to apparently pass from the cytoplasm through the plasmalemma and into the cell wall. Esterase activity was also shown to be present in association with the cell walls and Golgi bodies. It is possible that the particles containing esterases are in some way involved with the transport of these enzymes from the Golgi bodies to the cell walls where they may participate in the cell-wall changes.

We acknowledge partial financial assistance from the Science Research Council.

Bitensky, L. (1962). *Quart. J. micr. Sci.*, **103**, 205.

Gahan, P. B. & McLean, J. (1967). *Biochem. J.* **102**, 47P.

Gahan, P. B., McLean, J., Kalina, M. & Sharma, W. (1967). *J. exp. Bot.*, **18**, 151.

Holt, S. J. (1959). *Exp. Cell Res.*, **7**, Suppl. 1.

Livingstone, D. C., Coombs, M. M., Franks, L. M., Gahan, P. B. & Maggi, V. (1969). *Histochemie* (in the Press).