the elution profile obtained after hydroxyapatite chromatography.

Analysis of the products of limited digests of native DNA by Sephadex-gel filtration and by DEAE-cellulose chromatography indicated that the hydrolysis was almost exclusively endonucleolytic. The exonucleases from snake venom and bovine spleen (Razzell, 1963) were used to show that the oligonucleotides produced by the liver deoxy-ribonuclease bear a 5'-phosphate terminus.

The enzyme can be distinguished from the alkaline nuclease of the mitochondrial fraction by its preference for native DNA, its specificity for DNA and its activity in the presence of different bivalent cations. Preliminary experiments suggest a further distinction, in that the activity is associated with the nucleus rather than the cytoplasm and is located in the chromatin acidic protein fraction.

Beaufay, H., Bendall, D. S., Baudhuin, P. & de Duve, C. (1959). *Biochem. J.* **73**, 623.

Curtis, P. J., Burdon, M. G. & Smellie, R. M. S. (1966). *Biochem. J.* **98**, 813.

Higgins, G. M. & Anderson, R. M. (1931). *Arch. Path.* **12**, 186.

Laskowski, Sr. M. (1961). In *The Enzymes*, 2nd ed., vol. 5, p. 123. Ed. by Boyer, P. D., Lardy, H. & Myrbäck, K. New York and London: Academic Press Inc.

O'Connor, P. J. (1968). *J. Physiol.* **197**, 52P.

Razzell, W. E. (1963). In *Methods in Enzymology*, vol. 6, p. 248. Ed. by Colowick, S. P. & Kaplan, N. O. New York and London: Academic Press Inc.

Shugar, D. & Sierakowska, H. (1967). *Progr. Nucleic Acid Res. molec. Biol.*, **7**, 369.

A Simple Method for the Isolation of Very Large Numbers of Leaf Protoplasts by using Mixtures of Cellulase and Pectinase

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Takebe, Otsuki & Aoki (1968) described a method for the isolation of leaf protoplasts in which cells isolated from the leaf by prior treatment with pectinase (Macerozyme) are subsequently treated with cellulase (Onozuka P1500) to digest away the cell wall (cf. Cocking, 1960). Work in this Laboratory has now shown that this method can be greatly improved and made much simpler by adding a mixture of the cellulase and pectinase directly to the leaf material. Very large quantities of protoplasts can be isolated by using mixtures of these commercially available enzymes.

Pieces, approx. 4cm.², from fully expanded leaves of 50-70-day-old tobacco plants (*Nicotiana tabacum* var. *Xanthi*), from which the lower

epidermis had been removed (cf. Takebe *et al.* 1968), were treated with a mixture of pectinase [0.5% (w/v) Macerozyme, Kinki Yakult Manuf. Co. Nishinomiya, Japan, or 0.5% (w/v) Pectinase, Sigma Chemical Co. Ltd., St Louis, Mo., U.S.A.; or 5% (w/v) Pectinol R10, Rohm & Haas Co., Philadelphia, Pa., U.S.A.] and cellulase [5% (w/v) Onozuka P1500 from *Trichoderma viride*, Kinki Yakult Manuf. Co.; or 5% (w/v) Meicelase P, from *Trichoderma viride*, Meiji Seika Kaisha Ltd., Tokyo, Japan] in 20% (w/v) sucrose adjusted to pH 5.6 with 2N-HCl. After an initial vacuum infiltration for 15min. the pieces of leaf were incubated in this enzyme mixture for 4hr. without shaking. The incubation with the enzymes was terminated by gently swirling the incubation mixture, pouring it through a coarse wire gauze and washing with a modified White's medium containing 20% (w/v) sucrose (Pojnar, Willison & Cocking, 1967). The very large quantities of spherical green isolated protoplasts obtained were washed free from contaminating enzymes by repeated flotation in this medium.

These isolated leaf protoplasts are stable for several days in this medium but unless they are subsequently transferred to a more suitable cell-wall regeneration medium (cf. Pojnar *et al.* 1967) progressive bursting takes place, often with release of the large central vacuole.

These isolated leaf protoplasts should be particularly suitable for investigations of pinocytic uptake of viruses, and, after cell-wall regeneration, for elucidating early stages in the virus infection of isolated plant cells (cf. Cocking & Pojnar, 1969).

Cocking, E. C. (1960). *Nature, Lond.*, **187**, 927.

Cocking, E. C. & Pojnar, E. (1969). *J. gen. Virol.* (in the Press).

Pojnar, E., Willison, J. H. M. & Cocking, E. C. (1967). *Protoplasma*, **65**, 460.

Takebe, I., Otsuki, Y. & Aoki, S. (1968). *Plant & Cell Physiol.* **9**, 115.

The Partial Inhibition of Yeast Invertase by Iodine

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Iodine partially inhibits invertase (Euler & Landergren, 1922); cysteine, glutathione, ascorbic acid and sodium amalgam do not reactivate the enzyme (Myrbäck & Willstaedt, 1957). We have investigated the reaction of iodine with purified, homogeneous invertase (Waheed & Shall, 1967).

The extent of inactivation is dependent on the iodine concentration; maximal inhibition of about

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

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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.

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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).