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 deoxyribonuclease 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.

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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).

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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 (Myrback & 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