after 10 min. Phosphatase activity was measured by incubating cell-free solutions with *p*-nitrophenyl phosphate.

Low concentrations of the *Cytophaga* preparation caused complete conversion of cells from *S. cerevisiae* N.C.Y.C. 1006 and *S. chevalieri* N.C.Y.C. 394 into protoplasts within 90 min only in the presence of a thiol reagent. Dithiothreitol was more effective than 2-mercaptoethanol even at a final concentration in the medium of 12 mM. Pretreatment of the cells with the thiol before incubation with enzyme resulted in more rapid lysis when compared with cells incubated simultaneously with enzyme and thiol. This suggests, in agreement with the observations of other workers (Davies & Elvin, 1964; Bacon, Milne, Taylor & Webley, 1965), that the thiol modifies the yeast cell wall in a permanent manner, rendering it more susceptible to lysis.

A comparison of the properties of enzyme preparations from *Helix pomatia* and *Cytophaga* showed that, at the same concentration, protoplasts were produced at a similar rate from *S. cerevisiae* N.C.Y.C. 1006 and *S. chevalieri* N.C.Y.C. 394 in the presence of thiol. There was no action on *S. cerevisiae* N.C.Y.C. 239 (see Anderson & Millbank, 1966) or in the absence of thiol. It is therefore possible that both enzyme systems may have a similar mode of action on susceptible yeast cell walls.

Since the measured increase in phosphatase activity during incubation seemed to be coincident with the formation of protoplasts, it would appear that there was concomitant removal of the cell wall and liberation of a constituent enzyme.

In addition to *S. cerevisiae* N.C.Y.C. 239, exponential-phase cells from samples of 'baker's yeast' are also resistant to the action of the *Cytophaga* preparation and will not form protoplasts. Bacon *et al.* (1965), using the culture filtrate from *C. johnsonii*, were also unable to degrade the walls of living baker's yeast.

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Serum Acid Hydrolases in the Mucopolysaccharidoses

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In recent years, the activities of certain carbohydrate 'acid hydrolases' have been assayed in the tissues and blood of patients with Mucopolysaccharidoses (Öckerman, 1968; Van Hoof & Hers, 1968; Gordon & Feleki, 1970; Patel, Tappel & O'Brien, 1970). Most of these samples have been obtained from patients with Mucopolysaccharidosis types I, II and III as classified by McKusick *et al.* (1965).

In our studies, sera samples were collected from siblings with the clinical symptoms of type IV (Morquio syndrome), siblings with type VI (Maroteaux-Lamy syndrome) and unrelated children with type I (Hurler syndrome) disease. The activities of *N*-acetyl- β -glucosaminidase, *N*-acetyl- β -galactosaminidase, α -L-fucosidase and α -D-mannosidase in the sera were assayed in the presence of their *p*-nitrophenyl glycosides. 4-Methylumbelliferyl β -D-glucuronide and hyaluronic acid from bovine vitreous humour were used as substrates to determine β -D-glucuronidase and hyaluronidase activities respectively. There was a marked increase in activity of the *N*-acetyl- β -hexosaminidases and β -glucuronidase in type I sera, in accord with the results of Öckerman (1968) and Gordon & Feleki (1970), whereas in type VI sera values were in the upper range of normal, and there were no differences in type IV sera. Hyaluronidase activity was decreased in type IV sera. A considerable variation in α -fucosidase activity was found and no correlations could be made. Apart from these differences, the activities of other enzymes were within the normal ranges.

It is perhaps characteristic in Mucopolysaccharidosis type VI that no marked deviation from normal serum enzyme activities can be established. Studies by Van Hoof & Hers (1968) on liver lysosomal enzymes, suggesting only minor alterations from normal, may support this conclusion (see Spranger *et al.* 1970).

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Properties of Phosphoenolpyruvate Carboxylase Isolated from Maize Leaves

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Phosphoenolpyruvate carboxylase (EC 4.1.1.31) has been purified 100-fold from maize leaves in 25% yield. The purification method differs from that of Lowe & Slack (1971) and involves isoelectric focusing as the final step (pI 5.2–5.3). Specific activities of better than 40 U/mg of protein were obtained.

The enzyme was most stable in 1 mM-dithiothreitol at 15°C at pH 7.0. When glycerol was added to the enzyme solution to a final concentration of 50% (v/v) it increased its specific activity and helped to maintain its activity.

If purified enzyme was fractionated on an agarose column (Bio-Gel A1.5), two protein bands emerged: first a small band of low-activity protein (A) followed by a large band of lighter high-activity protein (B). If samples of band B material were re-run on the same column after 24 h storage at 15°C, two band were eluted at the same positions as bands A and B; after 5 days' storage the proportion of band A material had increased at the expense of band B material.

Band B material sedimented as an homogeneous protein in the analytical ultracentrifuge with an estimated $s_{20,w}^0$ value of $13.5 \pm 0.3S$ (possible variation in the slope of the extrapolation to zero concentration). The diffusion coefficient was estimated to be $3.77 \times 10^{-7} \text{ cm}^2 \cdot \text{s}^{-1}$ and the partial specific volume as $0.74 \text{ cm}^3 \cdot \text{g}^{-1}$. From these data a molecular weight of 340000 ± 8000 was calculated.

When a solution of band B material was diluted from 7 mg of protein/ml to 5.5 mg/ml, a second component was seen with a sedimentation rate corresponding to approximately half the molecular weight of the main component.

Treatment of band A material and band B material with sodium dodecyl sulphate followed by polyacrylamide-gel electrophoresis in the presence of this detergent showed a single component with similar mobility in each case. Comparison with the mobilities of protein standards indicated a molecular weight of approx. 160000 for the detergent-treated enzyme.

Phosphoenolpyruvate carboxylase from maize leaves appears to exist in several forms. The active

enzyme (B) has a molecular weight of approx. 340000. In concentrated solution (10–40 mg of protein/ml) this B form can aggregate to a larger, less active, form (A). On dilution or on detergent treatment the B form dissociates into probably two similar subunits.

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Carbonic Anhydrase: Multiple Forms in Plants

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Carbonic anhydrase (EC 4.2.1.1) catalyses the hydration of carbon dioxide to give HCO_3^- and H^+ ions:

It is present in the green parts of plants, and appears to be important for the efficient fixation of carbon dioxide (Graham & Reed, 1971). Iso-enzymes of carbonic anhydrase from animals can often be located after starch-gel electrophoresis by using their relatively weak esterase activity (Tashian, 1965). We could not detect any esterase activity associated with the carbonic anhydrase from the leaves of the angiosperm *Tradescantia albiflora* Kunth. We therefore devised a method to locate the enzyme after electrophoresis on polyacrylamide gels. The gels are surface-stained with Bromocresol Purple and exposed to carbon dioxide. H^+ ions produced by the enzyme-catalysed hydration of carbon dioxide change the colour of the indicator. The differential staining of the enzyme bands is a transient phenomenon, but further hydration is stopped by freezing the gels on solid carbon dioxide. At low temperatures solid solutions of Bromocresol Purple fluoresce brightly under u.v. light, the acid form yellow and the base form pink, and the position of carbonic anhydrase is therefore shown on the frozen gels as yellow bands against a pink background.

Extracts of leaves from higher plants were separated by electrophoresis through gradients of polyacrylamide (Margolis & Kenrick, 1968). All the extracts examined gave at least two major bands of carbonic anhydrase activity. Although it is

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