particular the exocellular phosphomannans from several species of *Hansenula* have been described (Jeanes & Watson, 1962; Slodki, 1962; Slodki & Boundy, 1970). One of these, *H. holstii* N.R.R.L. Y-2448, forms an essentially linear polymer with a mannose/phosphorus molar ratio 5:1. This report describes the results of our studies on the mannans from the related organism *H. holstii* N.C.Y.C. 560.

The yeast was grown in the medium described by Slodki, Wickerham & Cadmus (1961) at 30°C. Exocellular mannan was isolated in high yield from the cell-free medium. Mannan was extracted from the cells by the procedure of Peat, Whelan & Edwards (1961).

The phosphorus content of the exocellular mannan was found to vary, depending on the ages of the culture, the product after 1 day having a mannose/phosphorus molar ratio 3.8:1, which altered to 30:1 after 5 days. This variation was not observed with the cell mannan, which had a molar ratio 100:1 at both stages of growth.

Ion-exchange chromatography on DEAE-cellulose of mannans from the 5-day culture indicated that each contained at least two components with similar charge and, in addition, that there were two more highly charged species in the exocellular mannan.

Partial degradation by the acetolysis technique (Gorin & Perlin, 1956), followed by molecular-sieve chromatography on Sephadex G-15, converted the exocellular mannan into a mixture of mannose (2%), mannobiose (5%), mannotriose (13%), mannotetraose (29%) and a fraction of molecular weight greater than 5000 (51%). Digestion of the original polysaccharide with an α -mannosidase from *Arthrobacter* GJM-1 (Jones & Ballou, 1968) removed 75% of the mannose units. Acetolysis of the residual material produced a chromatographic pattern from which the high-molecular-weight fraction was absent. The product from the cell mannan contained only one fraction, which was eluted near the void volume of Sephadex G-15, suggesting that few (1→6)-mannopyranoside linkages were present. Although prior degradation with α -mannosidase removed 39% of the mannose units, no change in the acetolysis pattern was observed.

There was little difference in the oxidation of the two mannans with periodate, both reducing about 0.75 mol of periodate and forming 0.17 mol of formic acid/mol.prop. of mannose. In contrast, after α -mannosidase hydrolysis, the residue from the exocellular mannan required 1.8 mol of periodate to produce 0.9 mol of formic acid/mol.prop. of mannose, whereas the values for the cell mannan remained almost unchanged.

Consideration of these results suggests that both mannan preparations are structurally distinct, the exocellular mannan resembling the branched

structure normally associated with 'baker's yeast' (Stewart, Mendershausen & Ballou, 1968) rather than the structure proposed for *H. holstii* N.R.R.L. Y-2448 (Jeanes & Watson, 1962). It is also possible that each preparation may contain more than one type of mannan.

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Formation of Yeast Protoplasts by Using an Enzyme Preparation from *Cytophaga*

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Enzyme systems from the snail *Helix pomatia* (Anderson & Millbank, 1966) and various microbial sources (Villanueva, 1966) will form protoplasts from a number of yeasts. A preparation from *Cytophaga* that contains significant amounts of endo- β -(1→3)-glucanase activity (Manners & Paterson, 1966) attracted our attention as being a suitable source of enzymes for the study of yeast protoplast formation.

Exponential-phase cells of *Saccharomyces cerevisiae* N.C.Y.C. 1006 and N.C.Y.C. 239, and *S. chevalieri* N.C.Y.C. 394 were prepared by using the media as described by Darling, Theilade & Birch-Anderson (1969). Cells were removed by centrifugation and washed with 0.6 M-potassium chloride before suspending them in a suitable volume of 0.04 M-potassium phosphate buffer, pH 6.0, containing 1.2 M-potassium chloride. Generally the cell suspension (1 vol.) was shaken at 37°C with the enzyme preparation (1 vol.; 0.24% in distilled water) containing either 2-mercaptoethanol or dithiothreitol. The extent of formation of osmotically sensitive protoplasts was determined by diluting samples (0.4 ml) with distilled water (2.4 ml) and measuring the change in extinction at 660 nm

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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