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Observations on the Purity of some Yeast Glycogen Preparations

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Recent investigations by Bacon and his co-workers (Bacon & Farmer, 1968; Bacon, Farmer, Jones & Taylor, 1969) and by Manners & Masson (1969) have shown that yeast cell-wall preparations contain a soluble β -(1 $\rightarrow$ 6)-glucan in addition to the branched insoluble β -(1 $\rightarrow$ 3)-glucan. A survey of the literature shows a considerable variation in the specific rotation and β -amylolysis limits of various yeast glycogen preparations, and this is rather surprising in view of the generally accepted molecular structure of the polysaccharide (Manners, 1957). It is possible that this variation could be due, in part, to contamination of the glycogen preparations to differing extents with β -(1 $\rightarrow$ 6)-glucan, and we now describe experimental evidence to support this suggestion.

Baker's yeast was fractionated by the method of Trevelyan & Harrison (1956) to give the following fractions: trehalose, mannan and alkali-soluble glycogen, acetic acid-soluble glycogen, perchloric acid-soluble glycogen and residual β -(1 $\rightarrow$ 3)-glucan. The glycogen samples were then deproteinized by treatment with ice-cold 5% trichloroacetic acid solution.

Samples of the acetic acid-soluble glycogen and perchloric acid-soluble glycogen were partially hydrolysed (0.167M-sulphuric acid at 100°C for 30min). Paper chromatography showed the presence of glucose and maltosaccharides (degree of polymerization 2–5), but no other oligosaccharides. However, when the conditions for partial hydrolysis were altered to those that would be suitable for the β -(1 $\rightarrow$ 6)-glucan luteose, i.e. 1M-sulphuric acid at 100°C for 1h, the partial hydrolysates contained glucose and oligosaccharides with the chromatographic mobility of gentiobiose and gentiotriose.

The yeast glycogens were then treated with a purified amyloglucosidase preparation, which had no action on luteose but with rabbit liver glycogen gave 98.4% conversion into glucose. By using both glucose oxidase and Nelson-Somogyi reducing-power methods of analysis, the acetic acid-soluble

glycogen gave 78 and 79% conversion into glucose, and the perchloric acid-soluble glycogen gave 83 and 85% conversion respectively. The enzyme digests were then heated to inactivate the amyloglucosidase, dialysed to remove the glucose, concentrated and then partially hydrolysed with 1M-sulphuric acid at 100°C for 1h. The two hydrolysates both contained glucose and oligosaccharides having the chromatographic properties of gentiobiose, gentiotriose and gentiotetraose. These results show clearly that the glycogen preparations were contaminated with a significant amount of β -(1 $\rightarrow$ 6)-glucan.

Fractionation procedures of the type devised by Trevelyan & Harrison (1956) have been used in studies of the changes in carbohydrate composition that occur during the growth of yeasts (see, e.g., Trevelyan & Harrison, 1956; Suomalainen & Pfäffli, 1961; Chester & Byrne, 1968). The present work shows that these results can now only have a semi-quantitative significance, since they do not take into account the rate of synthesis of the β -(1 $\rightarrow$ 6)-glucan. The existence of the latter was, of course, not known when the fractionation procedures were carried out.

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The Cell and Exocellular Mannans from *Hansenula holstii* N.C.Y.C. 560

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Mannose-containing polysaccharides have been isolated from the cell walls of most yeasts. These may occur as homoglycans or in combination with other monosaccharides, phosphate and protein. Certain species will also produce exocellular mannans that may be conveniently isolated from the culture media after removal of the cells. In