Liberation of Invertase from Disintegrated Yeast Cells

Myrbäck[1,2] found that if baker's yeast was treated with ethyl acetate for 20 hr. at room temperature and then thoroughly washed with water on the centrifuge a large proportion of its invertase activity remained in the insoluble fraction even when this was stored for several months at room temperature. It has long been known[3] that only a small fraction of the total invertase can be removed from yeast cells by simple extraction procedures, some form of autolysis being needed to liberate the remainder. Myrbäck suggested that treatment with ethyl acetate removes from the cell the enzymes responsible for the autolytic release of the invertase, which therefore remains indefinitely in combination with insoluble cell structures.

We have confirmed the effect of ethyl acetate treatment, but have obtained additional evidence which indicates, contrary to Myrbäck's view, that yeast invertase does not occur in an insoluble form.

In our experiments we used baker's yeast from a factory at Libán, Czechoslovakia. 150 gm. was kneaded with 15 ml. redistilled ethyl acetate; it liquefied, and was then shaken for 20 hr. at room temperature. After dilution to 1,500 ml. with distilled water the insoluble fraction was separated and washed repeatedly on the centrifuge, the final wash being colourless and virtually devoid of invertase activity. The precipitate was suspended in water to a total volume of 150 ml., and when plated on wort-agar showed no sign of living cells. It was preserved at room temperature with chloroform.

The total invertase content of each batch of yeast was assumed to be the sum of the activities of the final ethyl acetate-treated suspension and of the first supernatant, measured by a polarimetric method. All results are expressed in units per gm. original pressed yeast, one unit being that quantity of enzyme which inverts 1 mgm. sucrose/min. from a 5 per cent solution at *p*H 4·7 (0·02 *M* sodium acetate buffer) at room temperature (20–22° C.).

Experiments were made with suspensions freshly prepared, or at most 7 days old. During this period not more than 5 per cent of the invertase activity passed into the suspending fluid. Samples were centrifuged and the precipitates crushed in the Hughes press[4]. For example, 10 ml. of a suspension was centrifuged (1,000*g*) on the day it was prepared and a known fraction of the precipitate transferred to the Hughes press, crushed at −30° C., and washed off the block and re-suspended with water so that 1 ml. corresponded to 0·5 gm. of original yeast. On centrifugation for 1 hr. at 35,000*g* a slightly opalescent supernatant was obtained. Its invertase activity was 57 units, compared with 65 units for the suspension before crushing and an estimate of 72 units in the original yeast.

This result suggests that the invertase in the intact yeast cell is enclosed by membranes which are not permeable to it, and which are not sufficiently damaged by ethyl acetate treatment to allow it to escape. It is conceivable that one of the membranes concerned is the glucan layer of the cell wall[5]. Treatment with crop juice of *Helix pomatia*[6], which is known to attack this glucan[7], at a final dilution of 1 : 10, liberated practically all the invertase (125 out of 130 units) from a sample of ethyl acetate-treated material in 8 hr. at 20° C. It has been suggested that invertase must lie near the surface of the yeast cell[3], and so it is possible that it lies between the glucan layer and a protein-containing layer[5]. This might explain the liberation of invertase by the action of papain[1].

It is unlikely that invertase is solubilized by autolytic processes during the ethyl acetate treatment, because the crushing of washed samples of living baker's yeast liberates a large proportion of the invertase in a water-soluble form. Further, on treatment with 0·1 volume of undiluted snail crop juice at room temperature some 50 per cent of the invertase of a sample of living yeast was liberated in 10 hr., while at least 90 per cent of the cells remained viable.

The preparations treated with ethyl acetate retain the outward shape of yeast cells but have obviously lost a great deal of their contents. After crushing, many of the cells show gross destruction, and, on prolonged treatment with snail digestive juice, holes appear in the cell walls. However, it would seem that often quite minor damage is sufficient to set the invertase free. Myrbäck's ethyl acetate-treated preparations might therefore provide a useful and sensitive system for the investigation of the nature of the yeast cell wall.

Two of us (E. E. B. and J. S. D. B.) wish to thank the Czechoslovak Academy of Sciences and Academician I. Málek, director of the Institute, for most generous hospitality which made our participation in this work possible.

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Aug. 6.

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Critical Effect of Oxygen Tension on Rate of Growth of Animal Cells in Continuous Suspended Culture

Its relevance to current animal virus and cell culture work prompts us to report the feasibility of and requirements for continuous culture of cells in suspension; full details will be given elsewhere.

Medium was pumped at a known rate from a reservoir to a culture vessel kept at 37° C. (20-l. 'Pyrex' aspirators), which overflowed at a constant level into a cooled receiver and was harvested once weekly. Culture vessel and receiver were gassed with mixed carbon dioxide, oxygen and nitrogen and magnetically stirred. Mechanical reliability was essential, as small breakdowns were rapidly lethal. The medium, a modified Earle's saline plus lactalbumen hydrolysate, amino-acids, yeast extract, vitamins, horse and calf serum, contained most components at their highest non-toxic level; these levels are probably not vital for continuous culture except for glucose (6–8 gm./l.). The limiting cell-density was probably not reached, but growth was very rapid under optimal conditions (defined below). Varying the gas-phase carbon dioxide and including bicarbonate kept the *p*H neutral; a transformed em-