The assistance of Messrs. J. Kuiper and T. O'Brien in critically examining this technique is gratefully acknowledged.

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Accumulation and Transformation of Sugars in Sugar Cane Stalks: Mechanism of Inversion of Sucrose in the Inner Space

PREVIOUS work¹⁻³ has shown that sucrose is the sole sugar transferred from the outer space to the inner space of sugar-cane storage tissue disks by an energy-coupled process. The outer space was identified tentatively as including the cytoplasm, and the inner space as the vacuolar volume.

By means of tracer studies, glucose and fructose which occur in relatively high concentrations in immature tissue were shown to arise from sucrose being inverted in the inner space. It was suggested that inversion may be mediated by a vacuolar invertase, and that an essential step in the maturation process would be masking or loss of invertase³.

Hartt⁴ found invertase in green-leaf and dry-leaf portions of sugar-cane stalks, while Fujii⁵ reported invertase in the rind at the tip of the stalk but absent from the storage tissue in the centre part of the stalk. Two enzymes which synthesize sucrose have been reported in sugar-cane—uridine diphosphoglucose-fructose transglycosylase⁶ and sucrose phosphorylase⁷. Both enzymes catalyse freely reversible reactions, which together with other enzymes could form systems for inverting sucrose.

The mechanism by which sucrose is inverted in storage tissue has been investigated. Initially the activity in crushed cells, juice and cell residues was compared. Radioactive sucrose was used as substrate and inversion followed by measuring the activity in glucose and fructose after chromatographic separation. Approximately 80 per cent of the activity in crushed cells was recovered in juice.

Hydrolysis of uniformly labelled sucrose by cane juice was carried out in the presence of excess unlabelled fructose, with the expectation that asymmetry of labelling in sucrose would develop if either of the sucrose synthesizing enzymes was functional in the system. No asymmetry was observed after 8 hr., during which more than 50 per cent of the substrate was hydrolysed (Table 1). In a similar experiment no labelled glucose was incorporated into endogenous unlabelled sucrose during a period in which 23 per cent of the sucrose was hydrolysed.

Experiments in which sucrose inversion by dialysed and undialysed juice was compared showed no lowering of activity following dialysis. The effectiveness of dialysis in removing small molecules was indicated by strong sucrose, glucose and fructose spots on paper chromatograms from undialysed juice whereas no sugars were detected in dialysed juice. Both the

Table 1. INCORPORATION OF FRUCTOSE INTO SUCROSE-U-¹⁴C DURING INVERSION

Sampling time (hr.)	Percentage of sucrose inverted	¹⁴ C in hydrolysis products of sucrose/ glucose/fructose	S.E.
0	0	1.08	0.07
1	14	1.12	0.02
3	31	1.08	0.03
8	58	1.06	0.02

1.0 ml. juice from immature storage tissues. Additions were 85 μ gm. sucrose-U-¹⁴C; 5,000 μ gm. unlabelled fructose. $T = 25^{\circ}\text{C}$. Sucrose in 0.1 ml. samples was separated on paper chromatograms, eluted and inverted with analytical invertase. Radioactivity in glucose and fructose moieties of sucrose was determined after separation on paper chromatograms. Results are means of quadruplicate determinations.

sucrose-synthesizing enzymes require dialysable co-factors. Lastly, no activity was detected in boiled juice. All these experiments were carried out with juice from immature storage tissue. The results show that an enzyme of the invertase type is responsible for sucrose inversion.

A comparison was made of the invertase activity in dialysed juice from storage tissue at different stages of maturity (Table 2). Relatively high activity was found in dialysed juice from very young internodes in which reducing sugar content is high. No activity was detected in juice from more mature internodes which contain large amounts of sucrose and practically no reducing sugars. The limit of detection of the method was about 10 enzyme units as defined in Table 2.

Table 2. INVERTASE ACTIVITY IN DIALYSED JUICE FROM STORAGE TISSUE OF DIFFERENT AGES

Internode No.	Internode diameter (cm.)	Internode length (cm.)	Enzyme content invertase units/ml. juice
5	1.3	2.5	374
6	1.9	10.1	108
8	3.2	14	nil
11	3.2	14	nil

Storage tissue was ground and centrifuged at 1,800g for 5 min. 0.7 ml. juice was removed and dialysed against distilled water for 3 hr. at 25°C . and with rapid stirring. The volume of the dialysed juice was noted, and 0.5 ml. samples taken for assay of invertase. The unit of invertase activity is arbitrarily defined as the amount of enzyme inverting 1.0 μ gm. sucrose/hr. in 0.83 per cent sucrose at 25°C . Assays carried out under toluene, with sucrose-¹⁴C as substrate. Radioactivity was determined in reducing sugars after separation on paper chromatograms. Reaction-rates for internodes 5 and 6 were constant over 27 hr.

The failure to detect invertase in immature storage tissue⁶ was probably due to insufficient sensitivity of the method used. The amount of enzyme present is very small but appears to be adequate to account for previous observations. In young tissue which contained 2.8 μM sucrose/25 disks the turnover time for the sucrose pool was 8.6 hr.³ This amount of tissue contained 0.30 ml. juice. Taking the figure of 374 invertase units/ml. juice from a young internode (Table 2), approximately 8 hr. would be required for the inversion of 2.8 μM sucrose. Hence the present results are in accord with the hypothesis that inversion of sucrose in the inner space (vacuole) of immature storage tissue is mediated by invertase, and the enzyme is inactivated during the maturation process.

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