THE SUGAR CONTENT OF CELL WALLS AND INTERCELLULAR SPACES IN SUGAR-CANE STEMS AND ITS RELATION TO SUGAR TRANSPORT

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Summary

In sugar-cane stems which contain large amounts of sucrose the concentration of sucrose in the volume external to the vacuoles was found to approach the concentration present in the vacuoles (20%). It was shown that this sucrose is situated mainly in the aqueous phase of the cell walls and intercellular spaces of the storage parenchyma cells.

It is suggested that the high concentrations of sucrose in this compartment contribute to the maintenance of the high levels of sucrose present in the vacuoles of parenchyma of cane grown under conditions of high sugar storage.

The presence of high concentrations of sucrose in the cell walls strongly favours the view that intercellular sugar transfer occurs mainly via the cell walls rather than via plasmodesmata.

I. INTRODUCTION

Experiments on sucrose accumulation by disks cut from mature stem tissue of sugar-cane revealed that many tissues were capable of maintaining only a 5% (w/v) gradient of sucrose between bathing medium and tissue. Influx of sucrose is made up of two components, an energy dependent process and diffusion (Hawker and Hatch 1965). Efflux was measured into an infinitely dilute solution. Since the sugar level in the vacuole of sugar-cane storage tissue cells may be as high as 23% (w/v) and may be maintained at this level for many months (Glasziou *et al.* 1965) it is possible that *in vivo* the concentration of sucrose outside the vacuole may exceed 15%.

It is generally considered that lateral movement of solutes through parenchyma cells of plant tissues occurs via the plasmodesmata (Webb and Gorham 1965) and that the concentration of metabolites in the aqueous phase of cell walls and intercellular spaces of tissue is low. Cormack and Lemay (1963) have presented radioautographs of sections of white mustard roots which had been fed ^{14}C-labelled glucose via cut cotyledons. Their results are consistent with sugars being translocated through intercellular spaces and cell walls in the root apex. From studies of a diphasic loss of reducing sugar from tissue slices Burg, Burg, and Marks (1964) concluded that the concentration of reducing sugar in the free space of whole McIntosh apples is approximately the same as that occurring in the vacuole. However, it appears that these results could be interpreted in terms of loss of sugar from cut and damaged cells in the first phase and cell bursting during washing in water in the second phase.

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In the present paper it will be shown that the concentrations of sugars in the aqueous phase permeating cell walls and intercellular spaces of intact sugar-cane stems approach the concentrations present in the vacuoles.

II. Materials and Methods

(a) *Sugar-cane Tissue*

Mature stem tissue of cv. Pindar, a commercial hybrid variety of sugar-cane, was used throughout. The plants were grown under natural light in glasshouses of a phytotron.

(b) *Sucrose Determination*

[^{14}C]Sucrose(U) was obtained from Radiochemical Centre, Amersham, England. Reducing sugars were determined by the method of Hoffman (1937) adapted to use with a Technicon Auto-Analyzer. Total sugars were measured by the same method following hydrolysis with dilute HCl. Sucrose, glucose, and fructose are the only sugars which contribute significantly to the total sugar content of sugar-cane.

(c) *Definitions*

(i) *Apparent Free Space of Cut Tissue.*—Apparent free space of cut tissue is defined in this paper as that part of tissue slices which comes into rapid diffusion equilibrium with a solution of sugar (Briggs and Robertson 1957). Rapid means about 30 min to 2 hr in 1–3-mm slices. This free space includes cut cells and cell walls, and may include part or all of the cytoplasm. It also includes any intercellular spaces which have become injected with liquid.

(ii) *Apparent Free Space of Intact Tissue.*—This is defined as that part of the apparent free space of cut tissue which exists in whole plants.

(d) *Measurement of Apparent Free Space of Cut Tissue*

(1) Transversely cut disks of mature stem tissue were prepared using a cork-borer and hand-microtome. All tissues used appeared turgid and microscopic examination revealed no plasmolysis. The disks were 18 mm in diameter and 1, 2, and 3 mm in thickness. Accurately weighed samples of about 1 g of disks were shaken gently in 1·5 ml juice extracted from similar tissue to which had been added a small volume of a solution of [^{14}C]sucrose(U) (1×10^6 disintegrations/min). Juice was used rather than water to avoid a large gradient in concentration between vacuole and medium and hence minimize the possibility of cells bursting. At frequent intervals aliquots (20 μl) of the bathing medium were spotted onto filter paper and the radio-activity counted with a Geiger–Müller counter (4·5% efficiency on paper) until the values became constant. Since accumulation of counts into the non-free space was negligible under these conditions reduction in the count rate was due to dilution of the label by the liquid in the free space of the tissue. Much of the apparent free space of cut tissue was due to cut cells as the 1-mm disks were only on an average six cells thick.

(2) Disks of tissue similar to those above were incubated for 2 hr in juice containing [^{14}C]sucrose(U). The disks were removed, blotted on moist paper, killed in two volumes of 95% ethanol, and extracted overnight in this solution. On removal of the disks the incubation medium was equally diluted with two volumes of ethanol. The amounts of radioactivity measured in aliquots of both the ethanolic disk extracts and the incubation medium provided an estimate of the volume of the apparent free space of cut tissue.

(3) Disks were incubated for 2 hr in 6% glucose, blotted, and the amount of glucose retained by the disks was measured. This method was used only when the level of endogenous reducing sugar in the tissue was negligible. Compared with the glucose in the free space only a negligible amount of glucose entered the vacuoles under these conditions.

(*e*) *Apparent Free Space of Intact Tissue*

For disks of constant diameter the apparent free space due to damage of cells at the edges will be proportional to the disk thickness. It will be shown that the apparent free space due to damaged cells on the upper and lower surfaces of the disks remains constant with increasing disk thickness.

The surface area (A) of a disk is given by

$$A = 2\pi r^2 + 2\pi rL, \tag{1}$$

where r = radius and L = thickness of disk. Volume (V) of free space due to cut cells is given by

$$V = 2\pi r^2 Z + 2\pi rL\ 0{\cdot}7Z, \tag{2}$$

where Z is a hypothetical factor indicating the depth of cell damage at the surfaces.

Experiments in which sugar loss from transverse and longitudinal sections was measured showed that damage was deeper in the transverse direction, this being attributed to the elongate shape of the cells. To correct for the disproportionate damage the factor of 0·7 is introduced.

For disks of radius 9 mm, substitution in equation (2) gives values of $9{\cdot}7x$, $10{\cdot}4x$, and $11{\cdot}1x$ for disks of 1, 2, and 3 mm thickness respectively, where x is equal to $18\pi Z$.

Hence the volume of the apparent free space of cut tissue, V_A, is given by:

$$V_A = 9{\cdot}7x + y, \qquad \text{for 1-mm disks}$$

$$V_A = 10{\cdot}4x + 2y, \qquad \text{for 2-mm disks}$$

$$V_A = 11{\cdot}1x + 3y, \qquad \text{for 3-mm disks}$$

where y is the volume of the apparent free space of intact tissue of a 1-mm disk.

From the above equations and the values obtained experimentally for apparent free space of cut tissue three estimates of y were obtained. Apparent free space could then be expressed as a percentage of the total volume of the tissue.

(*f*) *Volume of Gas in Tissue*

An internode of cane was placed in water, shaken to remove adhering air bubbles, and then vacuum infiltrated with water. The volume of gas displaced was measured by the apparent change in volume of water present.

The weight of the internode before and after infiltration also gave an estimate of the volume of gas in the stalk. The results from the two methods agreed.

(*g*) *Estimation of Volume of Cell Walls and Intercellular Spaces*

Microphotographs of transverse and longitudinal sections of mature stem tissue were prepared. The outline of all cell walls, intercellular spaces, and lumina of the vessels in the prints of transverse sections were separated from the vacuoles and weighed. In longitudinal sections only the outlines of the end cell walls were weighed. The walls, intercellular spaces, and vessel lumina were expressed as a percentage of the total volume of the tissue. Since it was impossible to get a clear print of every cell wall and to cut out the cell walls accurately the figure obtained was not an accurate one. The volume of the cytoplasm was ignored, firstly because of uncertainty as to whether the cytoplasm is included in the free space and secondly because in the mature tissue under examination it formed only a small portion of the total volume of the tissue.

(*h*) *Sugars in the Apparent Free Space of Intact Tissue*

Disks of tissue were cut, weighed, and washed in running tap water for 2 hr to remove sugars from the free space of cut tissue. Washing for 1 hr removed 96% of the sugar from the free space of the 3 mm disks and there was no further measurable loss of sugar after 90 min. Large pieces of similar tissue from the same internode were frozen in dry ice. While still frozen, samples of tissue were taken from well inside the original cut surfaces. This was to avoid using tissue in which sugars had become redistributed by diffusion from cut vacuoles. The frozen tissue and the washed disks were weighed and extracted in 70% ethanol. After the amount of sugars in the original tissue and washed disks had been measured the total amount of sugar diffusing from the apparent free space of cut tissue was found by subtraction. Using a set of equations similar to those already described the amount of either reducing sugar or sucrose in the free space of intact tissue was calculated.

(*i*) *Isolation and Estimation of Sucrose in Conducting Tissue*

Stems of sugar-cane were cut into approximately 35-cm lengths. The rind was cut from the upper 2 cm. The soft parenchyma was removed from the top tissue with fine forceps leaving the hard conducting strands standing like bristles on a brush. These were washed under running tap water for several minutes and then dried with paper tissues. Air pressure was applied to the lower end of the piece of cane and the small drops appearing at the ends of the conducting tissue were collected with a micropipette. Up to 1 μl of solution was collected and spotted directly onto Whatman No. 1 paper for chromatography using ethyl acetate–pyridine–water (8 : 2 : 1 v/v) as the developing solvent. Sucrose was located using *p*-anisidine

phosphate. The colour intensity of the sucrose spots was determined in a recording reflectance densitometer and compared with standard sucrose spots on the same chromatogram.

(*j*) *Statistical Analyses*

Each value given in the tables is the mean of several determinations and is presented with its standard error. Differences between means were tested for significance by Student's *t*-test.

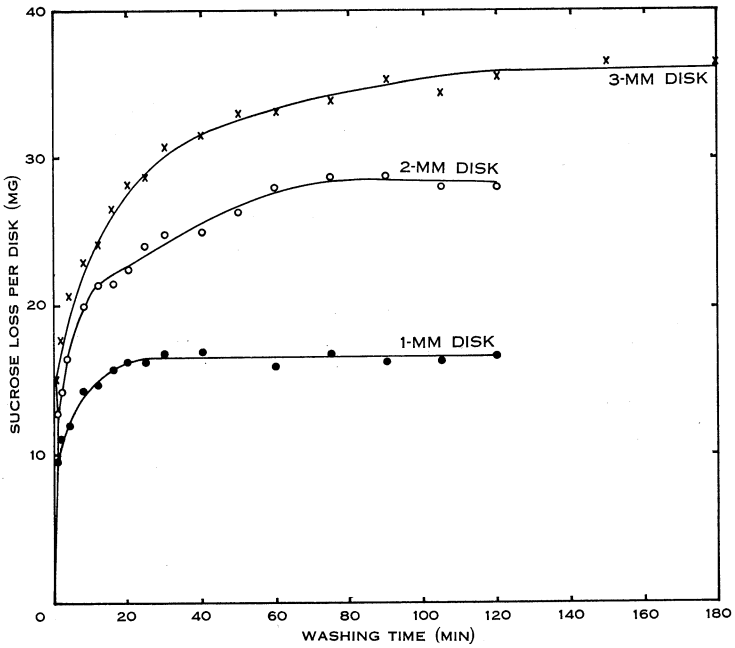

Fig. 1.—Loss of sucrose from disks of mature sugar-cane storage tissue 18 mm in diameter and 1, 2, and 3 mm thick. Disks were cut and immediately placed into water with gentle shaking. Samples of solution were analysed for sucrose.

III. Results

(*a*) *Volume of Apparent Free Space*

The volume of the apparent free space of intact tissue determined by the first two methods described above is shown in Table 1. The value obtained using microphotography, which included air in the intercellular spaces, was 18·4%.

(*b*) *Diffusion of Sucrose from Disks of Storage Tissue*

The amount of sucrose diffusing from disks as a function of time was determined by gently shaking samples of disks (20 g) in 100 ml water and assaying small aliquots of the solution for sucrose. The results for disks of 1, 2, and 3 mm thickness and 18 mm diameter are shown in Figure 1. After subtracting the amount of sucrose

released from cut cells the half times for the loss of the remainder of the sucrose were 2, 8, and 8 min for 1-, 2-, and 3-mm disks respectively. These values for 1- and 2-mm disks are consistent with the rate of loss of sucrose being diffusion from sheets. The half time for the 3-mm disks is lower than expected for a sheet and is probably due to disks of this size acting as open-ended cylinders (Phillip 1958).

TABLE 1

APPARENT FREE SPACE IN SUGAR-CANE STORAGE TISSUE

Method of Determination	Type of Free Space	Mean Free Space (as % of whole tissue volume)
Equilibration with [^{14}C]sucrose (U)	Liquid	13·6±0·9
Vacuum infiltration	Gas	4·1±0·4
	Total	17·7±1·0
From tissue microphotographs	Total	18·4±1·1

After all the sucrose had diffused from cut cells and the free space it was observed that the rate of efflux of sucrose from the vacuoles was very low (Fig. 1).

(*c*) *Concentration of Sucrose in the Apparent Free Space of Sugar-cane Storage Tissue*

Concentrations of sucrose (% w/v) in the apparent free space of stems of sugar-cane grown under different environments are shown in Table 2. Concentrations

TABLE 2

CONCENTRATION OF SUCROSE IN THE APPARENT FREE SPACE OF INTACT SUGAR-CANE STORAGE TISSUE

Environment	Mean Sucrose Concentration in Free Space* (15% free space)		Sucrose Concentration in Tissue (as % in juice)
18°C for 20 weeks	26·4%±1·3	***	18·2
30°C for 20 weeks	16·1%±1·7		12·4
18°C for 20 weeks	20·3%±0·9	***	17·9
18°C for 20 weeks then 30°C for 3 days	8·0%±2·2		17·0
18°C for 21 weeks	16·3%±1·3	**	19·7
18°C for 20 weeks then 30°C for 10 days	8·5%±1·4		18·2

* Each value is the mean of 12 determinations.
** Means significantly different at 1% level.
*** Means significantly different at 0·1% level.

were calculated on an apparent free space volume of 15%. Each value given in the table is the mean of 12 determinations (three made on each of four internodes of a stalk). For comparison the total amount of sugar present in the intact tissue is

expressed as the percentage in juice, i.e. the concentration (% w/v) in juice squeezed from macerated tissue. Cane growing at 18°C with 18·2% sucrose (in juice) had a concentration of 26% (w/v) sucrose in the apparent free space. Cane growing at 30°C with 12·4% sucrose (juice) had 16% sucrose in the apparent free space. The means are different at the 0·1% level. Cane which had been growing at 18°C was placed at 30°C. After 3 days at 30°C the apparent free space sucrose had dropped to 8% while plants remaining at 18°C had 20·3% sucrose in the apparent free space. The means are significantly different at the 0·1% level. After 10 days the values were 8·5% and 16·3% and are significantly different at the 1% level.

TABLE 3

CONCENTRATION OF SUGARS IN THE APPARENT FREE SPACE OF INTACT SUGAR-CANE STORAGE TISSUE

Environment	No. of Determinations	Reducing Sugar Concentration in Tissue (as % in juice)	Reducing Sugar Concentration in Free Space (% w/v)	Sucrose Concentration in Tissue (as % in juice)	Sucrose Concentration in Free Space (% w/v)	
30°C then 18°C for 4 days	24	4·3	2·3±0·3	6·7	5·4±0·7	
30°C then 18°C for 4 weeks	12	1·7	2·6±0·4	11·9	8·5±1·3	
30°C then 18°C for 8 weeks	12	0·05	—*	14·1	14·6±1·2	
30°C then 18°C for 13 weeks	12	0·05	—*	15·2	15·5±1·7	***
30°C then 18°C for 11·5 weeks then 34°C for 11 days	12	0·05	—*	10·9	2·9±0·6	***
30°C then 18°C for 14 weeks	24	0·05	—*	16·7	19·7±1·6	**
30°C then 18°C for 11·5 weeks then 34°C for 19 days	24	0·05	—*	13·0	13·3±1·5	**

* Not determined but $<0·05\%$.

** Means significantly different at 1% level.

*** Means significantly different at 0·1% level.

In another experiment using plants initially containing low concentrations of sucrose and relatively high concentrations of reducing sugar the concentrations of sugars in the apparent free space were determined as the sugar levels in the tissues changed. The results (Table 3) show that as the sucrose content of the juice increased the concentration of the sucrose in the apparent free space increased. The concentration of reducing sugar in the apparent free space was initially about 2% but fell to a low level after 8 weeks. On moving plants to 34°C after 11·5 weeks at 18°C decreases in the sucrose levels in the apparent free space again occurred.

(*d*) *Effect of Washing Disks in Mannitol*

In order to decrease the osmotic gradient between vacuole and washing medium, disks were washed in a solution of 6% mannitol instead of water. The results obtained for the concentration of sucrose in the apparent free space of cane grown under two different environments were not significantly affected by washing disks in mannitol although a consistent small decrease was observed in both types of tissue (Table 4).

TABLE 4

CONCENTRATION OF SUCROSE IN THE APPARENT FREE SPACE OF SUGAR-CANE STORAGE TISSUE AS DETERMINED BY WASHING DISKS IN 6% MANNITOL OR WATER

Each value is the mean of 12 determinations; n.s., means not significantly different

Environment	Disk Treatment	Sucrose Concentration in Tissue (as % in juice)	Sucrose Concentration in Free Space (% w/v)	
30°C then 18°C for 14 weeks	Washed in water	16·7	21·8±2·0	n.s.
	Washed in 6% mannitol	16·7	17·7±2·3	
30°C then 18°C for 11·5 weeks then 34°C for 19 days	Washed in water	13·0	15·2±2·2	n.s.
	Washed in 6% mannitol	13·0	11·1±2·1	

(*e*) *Volume of Apparent Free Space in Plants Grown at Different Temperatures*

Free space determinations were carried out on tissue from the last two plants listed in Table 3. The mean values were not significantly different (Table 5) and agreed with previous determinations on similar tissue (Table 1).

TABLE 5

APPARENT FREE SPACE IN SUGAR-CANE STORAGE TISSUE GROWN AT DIFFERENT TEMPERATURES

Environment	Method of Determination	No. of Determinations	Type of Free Space	Mean Free Space (as a % of whole tissue volume)
30°C then 18°C for 14 weeks	Equilibration with glucose	9	Liquid	14·4±2·0
30°C then 18°C for 11·5 weeks then 34°C for 19 days	Equilibration with glucose	9	Liquid	13·4±1·8

(*f*) *Concentration of Sucrose in Conducting Tissue Solution*

Conducting tissue solution was collected immediately after cutting (this was about 30 min after cutting by the time the stem had been prepared), or after standing 24 hr in a humid atmosphere at 22°C. A solution of 20% (w/v) sucrose was forced into some stems, and a sample was collected immediately and again after 24 hr. The results are shown in Table 6. Note that the concentration of sucrose in the solution was less than 10%. The conducting tissue supplied with 20% sucrose had only 6% present after 24 hr.

IV. Discussion

The sugar-cane stem tissue studied consists of approximately 92% storage parenchyma cells, 7·5% xylem and sclerenchyma, and 0·5% phloem. The vacuoles of the storage parenchyma cells constitute about 80% of the total tissue volume. The cytoplasm of these cells constitutes less than 2·5% of the total tissue volume (from measurements made on plasmolysed cells). From the results presented it is obvious that the bulk of the sucrose retained in disks washed in water is situated in the vacuoles of the storage parenchyma cells.

The determined values for apparent free space sugar concentrations often approach the concentrations present in the vacuoles. It is apparent from the following evidence that this sugar was in the free space in intact plants and was not a measure of sugar lost from vacuoles due to excessive cell damage during cutting or due to a

Table 6

CONCENTRATION OF SUCROSE IN SOLUTION FROM CONDUCTING TISSUE OF SUGAR-CANE STEMS

Environment	Sucrose Concentration (% w/v) in Solution	
	Immediately after Cutting	24 hr after Cutting
18°C	9·0±1·0	5·8±0·9
18°C with 20% (w/v) sucrose blown in	22·4±2·2	6·1±0·6
30°C	8·5±1·3	6·5±1·7
30°C with 20% (w/v) sucrose blown in	16·4±0·5	6·4±0·8

temporary break in cell membranes during washing of disks. If the extra sugar lost from thicker disks had been the result of damage to internal cells near the edges of the disks then the loss of sugar would not have followed the pattern shown in Figure 1. Had there been considerably more damage to the internal layers of cells in the 2- and 3-mm slices than anticipated, the value obtained for apparent free space would have been an overestimation. However, the value obtained in this way compares well with the value obtained by microphotography (Tables 1 and 5). This suggests that the assumption that only the surface cells were damaged during cutting was valid.

Evidence against a temporary break in membranes occurring was obtained by moving cane plants from a low temperature to a higher temperature. The rate of stem elongation of sugar-cane plants at 18°C is very low and sucrose is stored in the stem tissue previously formed at higher temperatures. When plants are moved from 18 to 30°C growth resumes and the sucrose content in the stem decreases (Glasziou *et al.* 1965). A drop from 17% sucrose to 6% sucrose in 5 weeks has been recorded. It was shown that respiration could account for only a small part of this decline in sugar content and hence that sucrose must have been translocated from the stem. It would be expected that the sucrose in the free space would be translocated first. Thus the level of sucrose in the free space would drop rapidly in cane moved from 18 to 30°C. The fact that the levels as determined by the present method did drop

under these conditions even though the vacuolar content of sucrose remained virtually the same (Tables 2 and 3) indicates that at least a large part of the sugar measured was in the free space and was not being lost from vacuoles during the washing of the disks. That there were no differences in the amount of sugar lost from cut cells of disks from plants grown at these different temperatures is shown by the apparent free space values in Table 5. Further evidence indicating that membranes remained intact during washing was obtained by reducing the osmotic gradient between vacuole and washing medium. This had little effect on the results obtained for apparent free space sugars (Table 4).

Having shown that there is a large amount of sucrose in a compartment external to the vacuoles in intact sugar-cane plants the question arises as to the location of this sugar. The concentration of sucrose in the xylem is less than 10% (Table 6). Were all the free space sucrose present in the phloem and cytoplasm of parenchyma cells (total less than 3·0% of tissue volume) the concentration of sucrose would be greater than 100% (w/v). The solubility of sucrose at 20°C is only 65% (w/v). The only logical conclusion is that a considerable portion of the free space sugar is located in the aqueous medium permeating the cell walls and intercellular spaces.

Apparently the high sugar concentration in the cell wall has two related functions *in vivo*. One is concerned with sugar storage. Hawker and Hatch (1965) have shown that the initial rate of active uptake of sucrose by mature tissue could maintain only a gradient of 5% (w/v) sucrose between the vacuole and free space. However, from information in the same paper on the rate of sucrose hydrolysis and the rate of glucose uptake by tissue slices it is apparent that the steady state concentration of reducing sugars in the free space could exceed 0·1% (w/v) in tissue containing high levels of invertase. Under these conditions a much larger gradient could be maintained. However, many of the tissues studied in the present paper were found to contain low levels of invertase and, as would be expected, also contained low concentrations of reducing sugars in the free space (less than 0·05%, Table 3). In these stems the high levels of sucrose in the vacuoles are probably maintained, in large part, by the high levels of sucrose in the free space.

The second function is concerned with intercellular sugar transfer. Evidence for this conclusion is given by the remarkable changes in the apparent free space sugar content of stem tissues by moving plants between environments which prevent stem growth and promote sugar storage, or promote both stem growth and removal of sugar from storage (Tables 2 and 3). In view of these findings the classical concept of the role of plasmodesmata mediating intercellular solute transfer through a cytoplasmic continuum must be questioned at least for sugar. The only direct evidence available favours the view that sugar transport occurs via the cell walls.

V. Acknowledgments

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