

THE PHYSIOLOGY OF SUGAR-CANE

III. CHARACTERISTICS OF SUGAR UPTAKE IN SLICES OF MATURE AND IMMATURE STORAGE TISSUE

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Summary

Sugar uptake by slices of sugar-cane storage tissue took place in two stages. The initial uptake reached an equilibrium within 1 hr, the level being proportional to the external sugar concentration, independent of the sugar, and unaffected by anaerobic conditions. This sugar diffused out rapidly when the tissue was placed in water. It was thus contained in the apparent free space, 10–20 per cent. of the tissue volume. The secondary uptake continued up to 60 hr at a slow, constant rate, 1–5 mg/g/day, independent of sugar concentration above 2.0 per cent., dependent on the sugar, and inhibited by anaerobic conditions. This sugar did not diffuse out when the tissue was placed in water. It was concluded that the secondary uptake was an active accumulation process.

The sucrose content of the tissue increased during accumulation, which occurred against a 10- to 200-fold concentration gradient. There was no starch synthesis, but accumulation was associated with a 30–40 per cent. increase in respiration. Sucrose was not hydrolysed prior to accumulation, and when slices accumulated sugar from a mixed solution, sucrose uptake inhibited glucose uptake. Internodes which were most active in storing sugar in the field gave the most actively accumulating preparations in laboratory experiments. Mature tissue slices showed little or no ability to accumulate sugars.

I. INTRODUCTION

Sugar transport† in animal tissues (LeFevre 1954, 1955) and sugar accumulation in bacteria (Davis 1956; Monod 1956) and yeast cells (Rothstein 1954) are well-documented processes. Harley and Smith (1956) and Harley and Jennings (1958) have demonstrated a probable sugar-accumulation process in lichen and in mycorrhizal tissues respectively. Studies on the general phenomenon of sugar uptake by higher plant tissues (Said 1941, 1950; Dormer and Street 1949; Said and Fawzy 1949; Said and Nada 1949; Street and Lowe 1950; Weatherley 1953, 1954, 1955; Porter and May 1955) have yielded only sketchy information on the specific process of sugar accumulation. Much of the necessary information is lacking because the contribution that respiration, starch synthesis, and particularly

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† The definitions of Kramer (1957) will be used, where “absorption” and “uptake” are general terms referring to the entrance of a substance into cells or tissues by any mechanism—diffusion, mass movement, or metabolic absorption; and where “accumulation” and “active transport” are terms describing an uptake which involves entrance to a cell and movement in a tissue against concentration gradients.

apparent free space (A.F.S.) uptake have made to the total sugar uptake has not been assessed. For example, the "active sugar uptake" process observed by Weatherley has since been shown (Pennell and Weatherley 1958) to be directed primarily towards polysaccharide synthesis rather than sugar accumulation *per se*. Even in the elegant experiments of Porter and May (1955) an unknown fraction of the radioactive sugars isolated from the leaf disks could have been unmetabolized molecules of the supplied sugar which had diffused into the A.F.S. In some cases (e.g. Said 1941, 1950) the amount of sugar taken up was such that, even assuming maximum possible A.F.S. absorption of the sugar, there was entry against a concentration gradient, indicating the operation of an active accumulation process.

There were reasons for expecting that sugar-cane tissues might be able to accumulate sugars. In extending studies on the respiration of sugar-cane (Bielecki 1958a) it was found that disks of sugar-cane tissue placed in aerated distilled water lost very little of their endogenous sugar to the water. Thus either the tonoplast is extremely impermeable to sugar movement or there is an accumulation mechanism in the cell which actively opposes the outward diffusional movement of sugar. The first is perhaps the simpler explanation, but raises the problem of explaining how the sugar originally became accumulated behind the impermeable tonoplast. Experiments were therefore carried out to examine the sugar-uptake process in sugar-cane tissue slices. An accumulation process is demonstrated, and some of its characteristics are established.

II. MATERIALS AND METHODS

Sugar-cane is essentially a giant erect or semi-erect perennial grass with solid stems, in which the parenchyma surrounding the vascular bundles has become adapted to efficient sugar storage. This storage tissue was used in the following experiments. The cane samples were cut from a commercial crop, cv. Pindar, grown near Ingham, N. Qld., and taken by air-freight to Sydney. The cane was washed, placed in an open-ended polythene bag to reduce moisture loss, and stored at 5–8°C. Canes could be kept in this way for at least 3 weeks without any detectable changes in the behaviour of the tissue samples; in practice the maximum storage period was 14 days. Mature internodes at least 6 months old were taken from near the base of the cane. Experiments (described below) showed that the most suitable immature internodal material was given by the internode which had an internal sugar concentration of 3–6 per cent., in which intercalary growth was ceasing or had just ceased, and which subtended the oldest green leaf or youngest dying leaf.

The nodal tissue was removed from the selected internodes, and the outer shell of protective sclerenchyma split off with a thin sharp blade. The parenchyma core (which contains numerous vascular bundles) was split longitudinally into quarters, and transverse quadrant-shaped slices, 1.2–1.7 mm thick, were cut by hand with a razor-blade. Disks of tissue, 1.1 cm in diameter and 1.2–1.6 mm thick, prepared by slicing cores of tissue taken with a cork borer, were used in one experiment only, since their preparation resulted in unnecessary wastage of tissue, and could have caused excessive compression damage. Furthermore, because cane storage

tissues were dense (sp. gr. 1.05–1.10) neither disks nor slices could be made to circulate in the aerating solution in the normal way. The regularly shaped disks tended to clump, hindering aeration, while the irregular quadrant-shaped slices remained separate, allowing the surrounding solution to circulate through them. Slices were washed for 15–20 hr in 5–7 changes of distilled water, blotted dry, and weighed into samples, generally 60 g (240 slices).

The disk/solution ratio was 1 : 2–1 : 3 (w/v) and the concentration of sugar (sucrose or glucose) supplied in the external solution was less than half the concentration of that sugar in the tissue, and generally about one-twentieth. The amount of sugar taken up by the tissue was estimated by measuring the decrease in sugar concentration of the external solution. Where sucrose was supplied (except

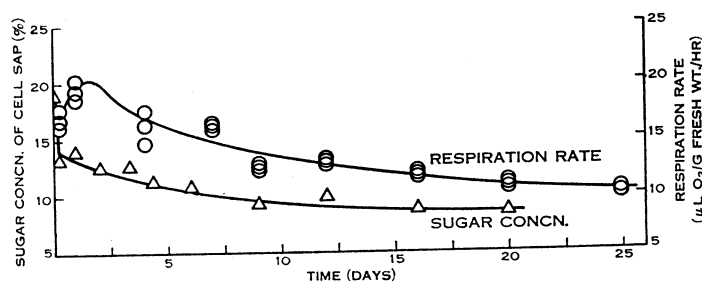


Fig. 1.—Change with time of respiration rate and sugar content of mature storage-tissue slices: $\circ$ respiration rate ($\mu\text{L O}_2/\text{g}$ fresh wt./hr); $\triangle$ percentage sugar concentration of cell sap. Samples of 15 disks (1.55 g) used. Triplicate samples used for respiration measurements (individual values plotted). Single samples used for sugar-concentration measurements.

Table 2) the changes in percentage dry weight of the solution were measured, it being assumed that all changes were due to sucrose uptake. Where glucose was supplied (except Fig. 8) the changes in concentration of reducing sugars were measured, using a modified Somogyi sugar reagent method (Somogyi 1945). A slow outward leakage of sucrose from the tissue which occurred during the course of the experiment was estimated by the dry weight method, but not the Somogyi method, which determined changes in reducing sugar concentration only. The uptake rate as measured by the dry weight method was therefore 5–10 per cent. lower than that measured by the Somogyi method.

The sugar content of the tissue samples (Fig. 1; Table 1) was estimated by measuring the refractive index of the expressed sap with an Abbé refractometer. Because sucrose is the only major soluble component of sugar-cane sap, this gives a reliable measure of sugar content. Each flask plus its contents (including aerator) was weighed initially and reweighed during the course of the experiment to allow for any evaporation of water from the solution, though with humidification of the air supply this precaution was generally not necessary. Experiments were conducted at room temperature.

At pH 5.5, 0.07M phosphate buffer increased the respiration rate of the tissue slices 15–40 per cent., while at pH 7.0 and 8.0 the respiration was increased up to 90 per cent. This did not appear to be a normal salt-accumulation respiration, but resembled the “uncoupled” respiration obtained with dinitrophenol (Bielecki 1958b). To avoid possible complications, therefore, buffers were not used, though the tissue itself effectively buffered the solutions at $\text{pH } 5.4 \pm 0.3$. No bactericide was used. Various methods were used to test for infection. The turbidity of the

TABLE 1

MOVEMENT OF SUGAR AGAINST A CONCENTRATION GRADIENT IN IMMATURE TISSUE SLICES

Expt. No.	Duration (hr)	Sugar Supplied	Final Concn. in External Solution (mg/ml)	Amount Absorbed in Second-stage Uptake (mg/g)	Ratio Absorbed/ External Sugar Concn.	Endogenous Sugar Concn. (mg/g)	Ratio Tissue/ External Sugar Concn.
XIII	100	Sucrose	10.76	23.79	2.21	52.7	7.12
			10.69	24.57	2.30		7.24
XV	30	Glucose	0.23	2.07	8.92	4.5–5.5	28–32
XVI	48	Sucrose	0.31	5.06	16.3	50–60	175–210
			0.26	5.33	20.5		210–250
XVII	43	Sucrose	1.02	3.04	2.98	62–67	64–70
		Glucose	0.87	3.36	3.76	9–11	14–17
		Fructose	0.45	4.18	10.75	7–9	25–30
XXIII*	24	Glucose	1.69	1.20	0.71		
			1.29	2.07	1.81		
			0.74	3.53	4.78		
			0.26	4.47	16.85		

* Internodes of various maturities used.

solution was estimated; or solutions and slices were stained and examined microscopically; or the respiration rate of the sugar solution was measured at the end of the experiment. Under the conditions of vigorous aeration used, bacterial infection did not become significant with immature slices in the first 24 hr, or with mature tissues over a period of 100 hr. The mature tissues appeared to inhibit growth of bacteria and fungi.

III. RESULTS

(a) *Respiration Drift and Sugar Loss in Mature Tissue Disks*

Mature tissue disks could be kept without infection or damage for periods of up to a month at 20°C, provided the distilled water was changed regularly. The pattern of respiration was determined by measuring the respiration rate of the

disks at intervals, using triplicate samples and standard Warburg manometry at 25°C (Fig. 1). The respiration rate fell steadily from 20 to 10 $\mu\text{l O}_2/\text{g}$ fresh wt./hr over the course of 550 hr. The respiration rate was thus of the same order as that of the single mature internodes (Bielecki 1958a).

The sugar concentration of sap extracted from the intact mature internodes was 19.0 per cent., but of sap extracted from disks 4 hr after cutting was 13.5 per cent. Two-thirds of the apparent dilution could be accounted for by the presence of water in the intercellular spaces (5 per cent. of the tissue volume as measured by vacuum injection) and in the volume of the cut cells (an estimated 17 per

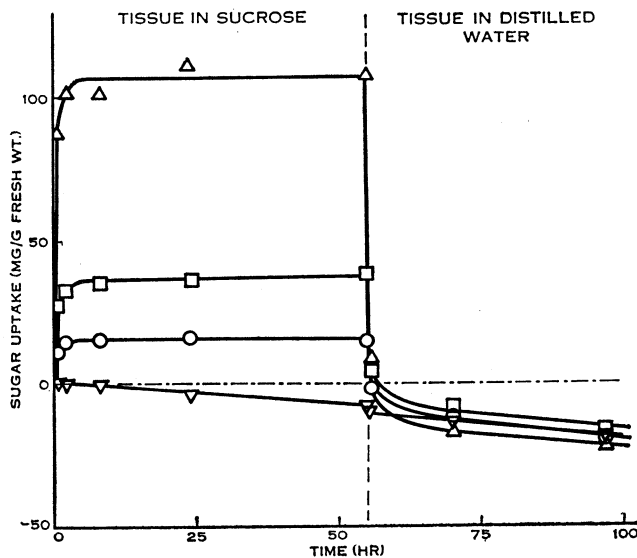


Fig. 2.—Sugar uptake by mature tissue slices from sucrose solutions of various concentrations, and its subsequent loss in distilled water: ∇ distilled water; $\circ$ 5 per cent. sucrose; $\square$ 10 per cent. sucrose; $\triangle$ 30 per cent. sucrose. At hour 55, tissue removed from sucrose solution, blotted, and placed in distilled water. Tissue sample weight 50.0 g; sucrose solution volume 150 ml; distilled water volume 150 ml.

cent. of the disk volume (Bielecki 1958b)). There may also have been some leakage from intact cells and some dilution by osmotic intake of water. Subsequent sugar losses were low, 0.2 per cent. per day (Fig. 1), respiration accounting for one-third and the remainder being lost either by leakage or occasional cell rupture.

(b) Sugar Uptake by Mature Tissue Slices

Slices of mature tissues from cane cut in July, with an internal sugar concentration of c. 10 per cent., rapidly took up sugars (sucrose and glucose), equilibrium being reached within the first hour, and the half-time of equilibration being 8 min. There was no further uptake of sugar over the next 54 hr. The amount of sugar taken up was exactly proportional to the sugar concentration of the

external solution. When the tissue was returned to distilled water, the absorbed sugar was rapidly and completely lost from the tissue (Fig. 2). Mannitol, generally regarded as being metabolically inert to most tissues, was taken up at exactly the same rate and to the same final equilibrium as sucrose (Fig. 3).

The effect of an anaerobic condition on the uptake of sugar by mature tissue slices was determined. The tissue sample was placed in a flask in distilled water, and aerated for 2 hr with oxygen-free nitrogen to remove any oxygen dissolved in the tissues. The flask was then inverted so that the water could be drawn off through the gas outlet, and the required sugar solution (pre-flushed with nitrogen)

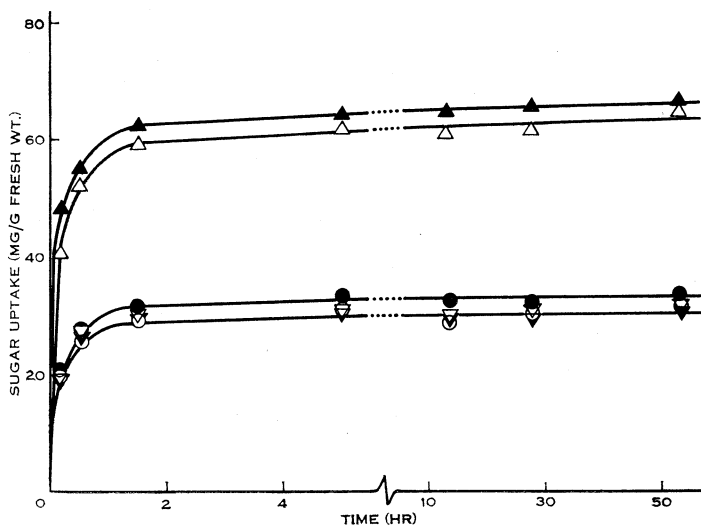


Fig. 3.—Sugar uptake by mature tissue slices in sucrose and mannitol under anaerobic conditions: ○ 10 per cent. sucrose, air; ● 10 per cent. sucrose, nitrogen; ▽ 10 per cent. mannitol, air; ▼ 10 per cent. mannitol, nitrogen; △ 20 per cent. sucrose, air; ▲ 20 per cent. sucrose, nitrogen. Tissue sample weight 60.0 g; solution volume 180 ml.

introduced without allowing any air to enter. The nitrogen flushing was then recommenced. The anaerobic conditions did not decrease the rate of sugar uptake or the amount absorbed at equilibrium (Fig. 3). These results all suggest that the sugar uptake observed was a passive uptake into the A.F.S. of the tissue.

(c) Sugar Uptake by Immature Tissue Slices

Sugar uptake by immature tissue slices took place in two stages. In the first stage, there was a rapid initial uptake of sugar which ceased within an hour (half-time *c.* 8 min). The amount of sugar taken up in this stage was proportional to the concentration of the external solution (Fig. 4). This first stage in immature slices was identical to the complete uptake pattern in mature slices, and can be regarded as due to passive sugar movement into the A.F.S.

In the second stage there was a slow uptake of sugar which continued at a constant rate for at least 20 hr and up to 60 hr. The rate of uptake was independent of sugar concentration above 2 per cent. (Fig. 4). When dilute sugar solutions (0.25 per cent.) were supplied to the tissue, the amount of sugar taken up in this second stage (exclusive of A.F.S. sugar and endogenous sugar) was such that there must have been a 2- to 20-fold concentration of sugar in the tissue from the external solution (Table 1). The second stage of uptake can therefore be regarded as an accumulation of sugar into the osmotic volume of the tissue by a metabolic process.

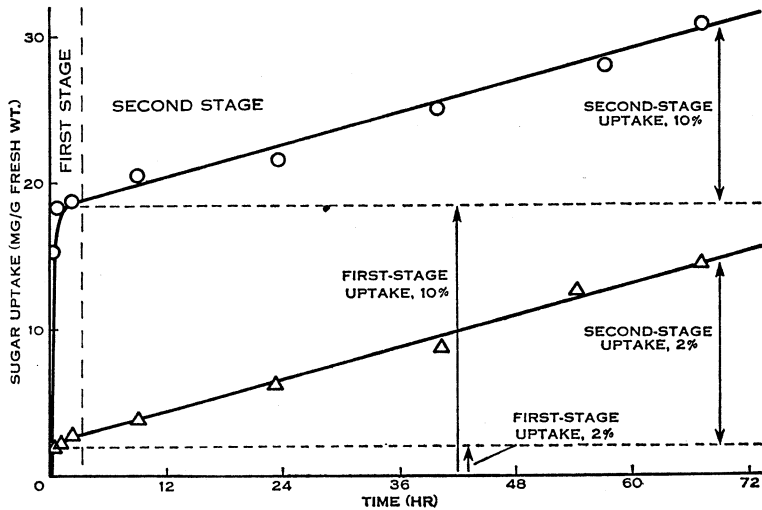


Fig. 4.—Sugar uptake by immature tissue slices from sucrose solutions of various concentrations: Δ 2 per cent. sucrose; $\circ$ 10 per cent. sucrose. Tissue sample weight 35 g; solution volume 125 ml. The first-stage (passive) uptake is proportional to the sugar concentration of the external solution, and the second-stage (accumulation) uptake is largely independent of concentration.

In solutions of mannitol the first stage of rapid uptake occurred, but the second stage of slow accumulation was practically absent (Fig. 5). When tissue slices which had previously been allowed to take up sugar from mannitol and sucrose solutions were blotted and returned to distilled water, sugar equivalent to the first-stage (A.F.S.) uptake was lost from the tissue to the water; while sugar equivalent to the second-stage (accumulation) uptake remained in the tissue (Fig. 5). The sucrose lost from the A.F.S. was then slowly taken into the tissue again by the continuing operation of the accumulation mechanism, at the original rate of accumulation, until the final equilibrium was established. At equilibrium, the endogenous sugar concentration was 8.1 per cent. (0.9 per cent. having entered the tissue by accumulation from the solution) and the solution concentration 0.017 per cent. The tissue which had been in mannitol re-accumulated the lost sugar only slowly. At least a part of the material which was re-accumulated may not

have been mannitol but sucrose and glucose, present in the solution through "leakage" of endogenous sugars from the tissue during the course of the experiment.

(d) *Effect of Anaerobiosis on Sugar Uptake in Immature Tissues*

When slices of immature tissue immersed in dilute sugar solutions were flushed with oxygen-free nitrogen instead of air, the rapid first-stage uptake was not affected, but the second-stage uptake was either greatly reduced or completely stopped (Figs. 6 and 7). Where the uptake was completely stopped, sugars already accumulated by the tissue leaked back into the solution (Fig. 7). When the accumulating sugar was glucose a considerable amount of sucrose appeared as the leaking

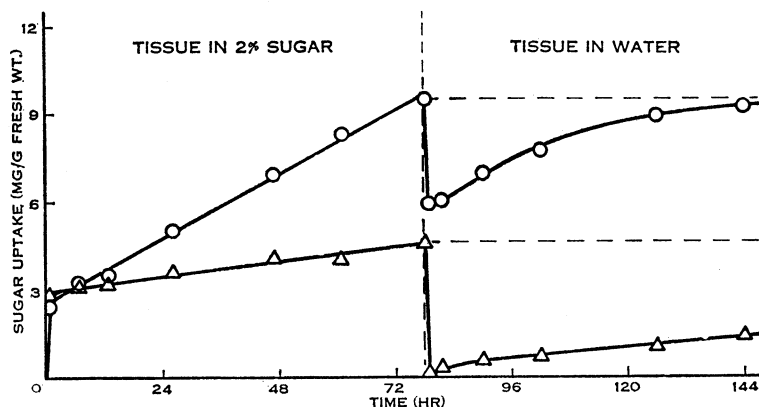


Fig. 5.—Uptake of sucrose and mannitol by immature tissue slices and their subsequent loss in distilled water: ○ 2 per cent. sucrose; △ 2 per cent. mannitol. Tissue sample weight 40.0 g; sugar solution volume 120 ml. At hour 78, tissue sample weight 40.6 ± 0.2 g; water volume 120 ml. The almost complete loss of mannitol to the water indicates that most of this sugar was held in the A.F.S. The volume of the A.F.S. must have increased during the course of the experiment, probably through cells bursting. Only part of the sucrose taken up can have been in the A.F.S. since only 35 per cent. was lost when the tissue was placed in distilled water, and 85 per cent. of this was taken up again into the tissue.

sugar, and when sucrose was supplied, some hexose sugar also leaked from the cells. When the tissue was aerated in distilled water to remove any products of anaerobic respiration then returned to fresh sugar solution and aerated with air, sugar accumulation proceeded at the original rate. The inhibition of uptake and induction of leakage by anaerobiosis were therefore completely reversible (Fig. 7).

(e) *Comparative Uptake Rates of Various Sugars in Immature Tissues*

Comparative tests of accumulation were made on fructose, glucose, sucrose, glucose 1-phosphate, and mannitol. In 0.25 per cent. solutions, fructose was accumulated at a rate similar to or slightly higher than that of glucose. Glucose was accumulated at a rate generally 25 per cent. higher than that of sucrose on a weight basis, and more than double on a molar basis (Fig. 8). However, when glucose and sucrose were supplied to the tissue together, glucose accumulation appeared to be

largely suppressed at the expense of sucrose accumulation (Table 2). The total uptake of the two sugars was more than that of either singly on a weight basis, but the same as the uptake of glucose alone on a molar basis.

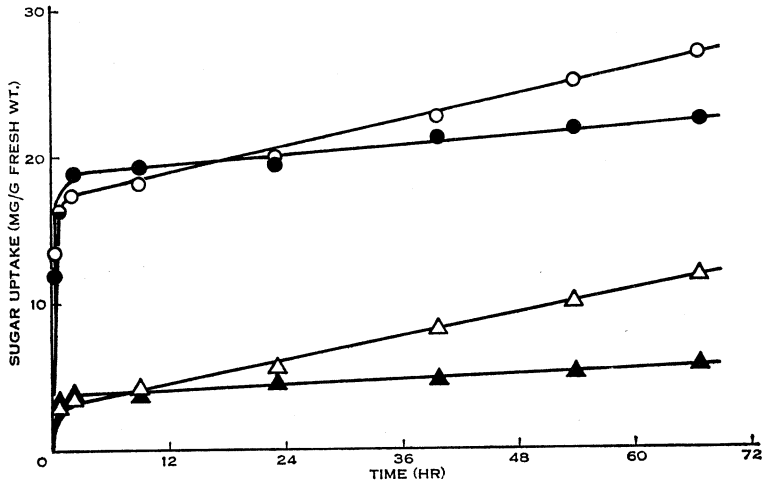


Fig. 6.—Effect of anaerobic conditions on sucrose uptake by immature tissue slices: $\triangle$ 2 per cent. sucrose, air; $\blacktriangle$ 2 per cent. sucrose, nitrogen; $\circ$ 10 per cent. sucrose, air; $\bullet$ 10 per cent. sucrose, nitrogen. Tissue sample weight 25.0 g; solution volume 75 ml.

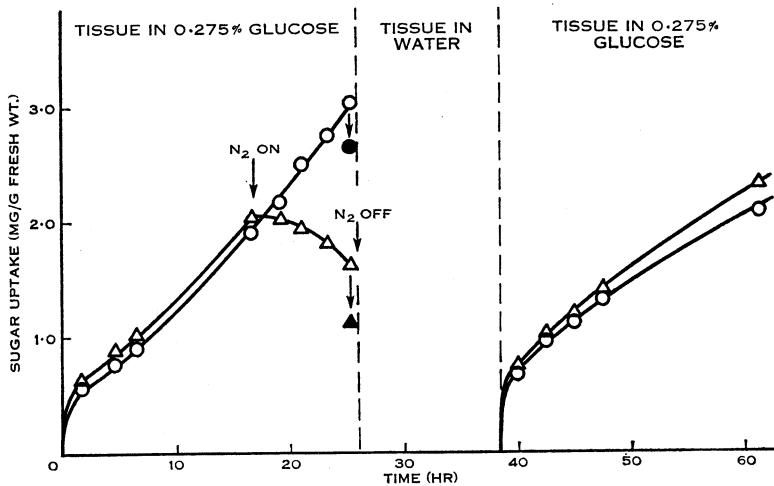


Fig. 7.—Reversible nature of the effect of anaerobic conditions on glucose uptake by immature tissue slices: $\circ$ 0.275 per cent. glucose, aerated with air; $\triangle$ 0.275 per cent. glucose, aerated with air till hour 17, then with nitrogen till hour 26, thereafter with air. Both samples placed in distilled water at hour 26 and washed till hour 38, then returned to 0.275 per cent. glucose solution. Tissue sample weight 110 g; glucose solution volume 240 ml; wash volume four changes of 350 ml. $\bullet$ Sucrose leakage from tissue, control. $\blacktriangle$ Sucrose leakage from tissue, anaerobic sample.

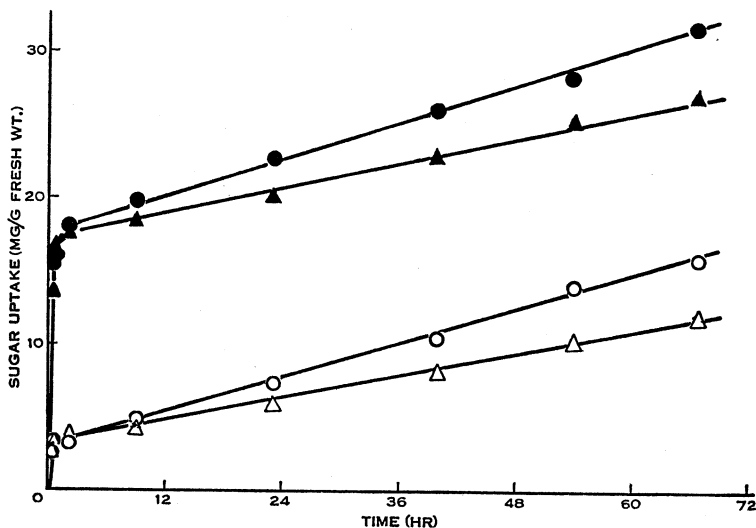


Fig. 8.—Comparative rates of uptake of sucrose and glucose by immature tissue slices: Δ 2.0 per cent. sucrose; $\circ$ 2.0 per cent. glucose; $\blacktriangle$ 10.0 per cent. sucrose; $\bullet$ 10.0 per cent. glucose. Tissue sample weight 25.0 g; solution volume 75 ml.

TABLE 2

RATE OF ACCUMULATION OF SUCROSE AND GLUCOSE FROM SIMPLE AND MIXED SOLUTIONS
Sugar concentrations given as mg/g tissue/hr. Values in parenthesis are these concentrations expressed as $\mu\text{M/g}$ tissue/hr

		Solution Supplied to Tissue		
		Glucose (2.5 mg/ml)	Sucrose (2.5 mg/ml)	Glucose plus Sucrose (each 2.5 mg/ml)
Glucose	Uptake	+0.200 (1.11)		
	Leakage†		−0.018 (0.10)	+0.078 (0.43)
Sucrose*	Uptake		+0.178 (0.53)	+0.194 (0.56)
	Leakage	−0.020 (0.06)		
Net sugar uptake		+0.180 (1.05)	+0.160 (0.43)	+0.272 (0.99)

* Changes in glucose concentration measured by changes in reducing sugars; net sugar uptake measured by changes in total (reducing plus non-reducing) sugars; and changes in sucrose concentration estimated by difference. Tissue sample weight 80 g, solution volume 160 ml.

† Part or all of this sugar could have come from hydrolysis of sucrose in the solution; but results of other experiments indicate that most came from leakage.

These results indicated that glucose might not be accumulated as such. The possibility was considered that sugar uptake might occur through glucose 1-phosphate. Glucose 1-phosphate was not accumulated under normal experimental conditions (Fig. 9). No significant hydrolysis occurred during the experiment. Phosphate ion by itself ($2 \times 10^{-3}M$, pH 5.5) did not increase the uptake of glucose. Sugar-cane tissues showed little tendency to accumulate mannitol.

(f) *Fate of Accumulated Sugars*

Starch formation could account for part or all of the sugar taken up. Immature tissue slices were tested for starch, before and after a period of accumulation, by perchloric acid extraction and an iodine colour test. No trace of starch

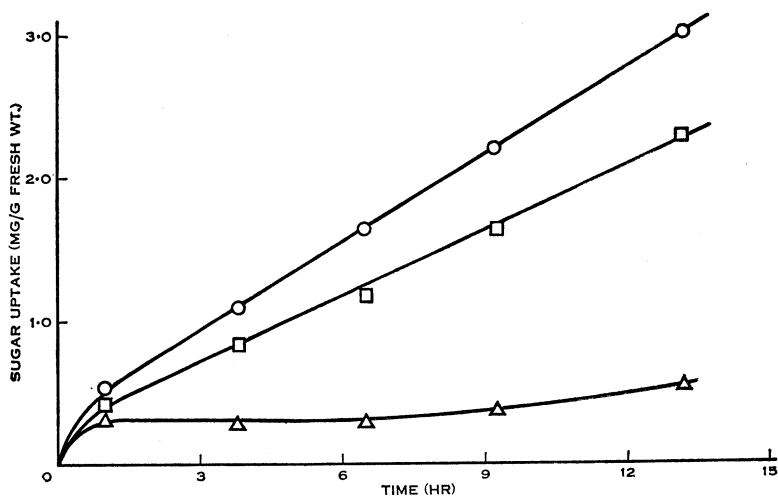


Fig. 9.—Comparative rates of uptake of sucrose, glucose, and glucose 1-phosphate by immature tissue slices: $\square$ 0.225 per cent. (6.3 mM) sucrose; $\circ$ 0.225 per cent. (12.5 mM) glucose; $\triangle$ 0.326 per cent. (12.5 mM) potassium glucose 1-phosphate. Tissue sample weight 80 g; solution volume 160 ml.

could be demonstrated in either sample. Microscopic inspection of stained sections also failed to reveal any starch, though sugar accumulation equivalent to 1 per cent. of the fresh weight had occurred.

In many tissues, addition of sugars causes an immediate rise in the respiration rate as the substrate is utilized. Part or all of the sugar taken up by sugar-cane could have been used in this way. Also, accumulative processes require energy to transport materials against a gradient; and therefore an increase in respiration similar to the "salt respiration" increase (Robertson and Wilkins 1948) would be expected to follow addition of sugars to immature sugar-cane tissue slices. Respiration rates of such slices were measured by conventional Warburg manometry before and after addition of glucose, sucrose, and mannitol, and compared with the accumulation rate of the sugar (Table 3). Accumulation of sugar was found to be associated with a 30–40 per cent. increase in respiration. The loss of sugar

in increased respiration accounted for 12 per cent. of the sugar taken up in the tissue.

An attempt was made to find whether the sugar disappearing from the external solution could be recovered as sugar from the tissue slices. Three paired samples of tissue were used: (i) tissue aerated for 103 hr in 2 per cent. sucrose, then washed in four changes of distilled water over 3 hr to remove the freely diffusible sugar held in the A.F.S.; (ii) tissue aerated for 49 hr in distilled water, then for 54 hr in 2 per cent. sucrose, then washed as above; (iii) tissue aerated for 100 hr in distilled water, then for 3 hr in 2 per cent. sucrose, then washed as above. The sucrose uptake was followed by measuring the dry weight changes in the external solution. At the end of the experiment, after the tissue samples had been washed

TABLE 3
COMPARISON OF "ACCUMULATION RESPIRATION" RATE AND SUGAR ACCUMULATION RATE

	Sugar Supplied to Tissue		
	Sucrose	Glucose	Mannitol
Initial respiration rate ($\mu\text{l O}_2/\text{g/hr}$)	46.0	48.5	48.0
Respiration rate in sugar ($\mu\text{l O}_2/\text{g/hr}$)	60.0	68.0	51.5
Respiration increase ($\mu\text{l O}_2/\text{g/hr}$)	14.0	19.5	3.5
Respiration increase as sugar loss ($\mu\text{g/g/hr}$)	17.8	26.2	4.4*
Sugar uptake ($\mu\text{g/g/hr}$)	148.0	189.0	20.8

* Assuming mannitol respired.

in distilled water, each sample was homogenized in a blender with 70 per cent. ethanol, the slurry filtered, and the residue extracted twice more with 70 per cent. ethanol. This procedure left less than 0.3 per cent. of the sugar in the tissue. Aliquots of the tissue extracts were analysed for sugar content by the Somogyi method, and the increase in sugar content through each period of accumulation was calculated by difference (Table 4). Less sugar appeared in the tissue than was lost from the solution. The 8 per cent. discrepancy probably represents the amount of sugar used in maintaining the accumulation respiration.

(g) *Effect of Tissue Maturity on Sugar Uptake*

The cane variety Pindar is a vegetatively propagated clone, and under favourable conditions crop growth may be so regular that it is possible to select uniform samples of internodes of various maturities. Eight closely matched cane tops were selected and four samples of tissue were cut from each cane top. In sugar-uptake

experiments it was found that the older the tissue the greater the first-stage uptake and the less the rate of uptake in the second stage (Table 5).

TABLE 4
COMPARISON OF SUCROSE UPTAKE AS MEASURED BY LOSS IN DRY WEIGHT OF EXTERNAL SOLUTION AND INCREASE IN TISSUE SUGAR CONCENTRATION

Absorption Period* (hr)	Sugar Uptake as Loss from External Solution* (mg/g) (mg/g/hr)		Sugar Uptake as Appearance in Tissue		
			Reducing Sugar (mg/g)	Non-reducing Sugar (mg/g) (mg/g/hr)	
51	11.9	0.234	0.1	11.4	0.224
	11.4	0.224	0.5	11.0	0.216
100	23.8	0.238	0.0	23.3	0.233
	24.6	0.246	0.0	22.0	0.220

* For sugar appearance in tissue, absorption period derived from (ii) minus (iii) and from (i) minus (iii)—see text, p. 214. For sugar loss from external solution "zero time" taken 3 hr after sugar solution added to (ii) and (i); thus making time intervals the same for the two methods and avoiding making uptake measurements until after diffusional, A.F.S. uptake complete.

At certain times of the year, even mature tissues could be shown to accumulate sugars. In March (the middle of the growing season) mature internodes (over 6 months old, from the base of the stem) had a low endogenous sugar content,

TABLE 5
INTERNODE AGE AND SIZE AS RELATED TO RATE OF SUGAR ACCUMULATION

Group	Phase of Growth of Internode	Mean Weight (g)	Mean Length* (cm)	A.F.S. (%)	Rate of Sugar Accumulation (mg/g/hr)
1	At final width, elongation starting	16.0 ± 2.4†	3.00 ± 0.43†	10	0.270
2	Elongation active	22.1 ± 2.9	4.30 ± 0.44	10	0.174
3	Elongation finishing	34.0 ± 3.9	6.61 ± 0.67	15	0.116
4	Elongation complete	47.1 ± 3.5	8.85 ± 0.44	19	0.084

* The internodes used were smaller than is usual at this stage of development.

† Standard deviation.

c. 10 per cent. Tissue slices from these internodes accumulated sucrose from a 0.5 per cent. solution at a rate one-third that of the immature tissue (Table 6).

IV. DISCUSSION

In mature sugar-cane tissue slices, the sugar uptake had the characteristics of a non-metabolic (diffusional) uptake into the A.F.S. (Briggs and Robertson 1957). Sugar uptake reached an equilibrium within an hour, the equilibrium was closely proportional to the concentration of the sugar solution supplied, independent of the nature of the sugar, and unaffected by anaerobic conditions: the sugar taken up was completely and rapidly released from the tissue when the tissue was placed in distilled water. From the amount of sugar taken up at equilibrium, the percentage A.F.S. of the tissue could be calculated, and was found to be 15–21 per cent. This is of the same order as has been found for other plant tissues. The volume of the A.F.S. appeared to be greatest in the tissues with the highest proportion of cell wall material. This ranged from 11 to 17 per cent. of the tissue

TABLE 6
ACCUMULATION RATE IN TISSUE SLICES AS RELATED TO INTERNODE AGE AND MATURITY

Internode Maturity	Month	Sugar Content (%)	Storage in the Field	Accumulation Rate <i>in vitro</i> (mg/g/day)
Immature	December	4	Active	4.30
Immature	March	4	Active	4.75
Immature	July	5	Active	4.15
Maturing	July	8	Medium	2.95
Mature	July	16	Little or none	<0.30
Mature	March	10	Slow	2.10

fresh weight, as estimated by tissue fragmentation and dry weight analyses. The vascular bundles formed about 7 per cent. of the tissue volume: part of this tissue was non-living, and would have contributed to the A.F.S. Cut cell surfaces and intercellular spaces, 3–6 per cent. of the tissue volume as estimated by water injection, were sufficient to account for the remainder of the A.F.S. volume. The cytoplasm was too thin to be distinguished microscopically from the cell wall in an unfixed section, so the volume of the cytoplasm was probably less than 3 per cent. of the cell volume, and too small to determine whether or not it formed part of the A.F.S.

In the immature tissue slices, the first stage of uptake also showed all the characteristics of an A.F.S. uptake: the uptake was rapid, independent of the nature of the sugar and proportional to the sugar concentration, and unaffected by anaerobic conditions; and when the tissue was returned to distilled water, sugar equivalent in amount to the first-stage uptake was rapidly released from the tissue. From the amount of sugar taken up in the first stage, the percentage A.F.S. of this tissue was found to be 10–18 per cent.

The second stage of sugar uptake in immature tissue slices had different characteristics: the uptake was slow, continued for a long time, was dependent

on the nature of the sugar but more or less independent of its concentration, and was affected markedly by anaerobic conditions; the sugar taken up in this stage was not released when the tissue was placed in distilled water. The amount of sugar taken up in this stage was such that the sugar from the external solution was increased in concentration 2–20 times in passing into the tissue. If the endogenous sugar concentration of the tissue, 3–6 per cent., is taken into account, it can be shown that sugar movement occurred against a 10- to 200-fold gradient (Table 1). The second-stage uptake therefore had the characteristics of an active accumulative process. The two-stage uptake pattern of sugars in sugar-cane is closely analogous to that observed for salt (ion) uptake in other plant tissues (Hope and Stevens 1952; Epstein 1955; Kramer 1957).

Since the sugar taken up in the second stage could not be recovered by rinsing the tissue in water, it must either have been transformed into insoluble compounds, or accumulated behind a permeability barrier. Starch did not occur, there was no sign that any cell multiplication or cell expansion had taken place, and most of the sugar taken up could be recovered as such by alcohol extraction of the tissue. The observed uptake of sugar must therefore have occurred through a true accumulation process.

One characteristic of accumulation processes is their frequent discrimination between closely related molecular structures. Opinion is divided as to whether sucrose is taken up as such into plant tissues or whether it must first be hydrolysed by the tissue. Since it is often the cell surfaces exposed by cutting that are responsible for the hydrolysis (Hassid 1958) the apparent dependence of uptake on hydrolysis may sometimes be an artifact (see Harley and Smith 1956). Though sucrose was rapidly accumulated by sugar-cane tissue there was negligible hydrolysis in the external solution, the traces of hexose which occurred apparently coming by slow diffusion from the cell vacuoles or by occasional rupture of cells. Some support to the theory that sucrose was taken up as such is given by the observation that when tissue was allowed to accumulate in sucrose, the sucrose content of the tissue increased while hexose remained constant. The rate of sugar accumulation from a solution of 0.25 per cent. glucose + 0.25 per cent. sucrose was 1.5 times as great as from a solution of 0.25 per cent. glucose alone (Table 2); this also suggests that sucrose is absorbed as such.

The results of these experiments do not provide much information on the mechanism of the sugar-accumulation process. As has been suggested for amino acid accumulation (Birt and Hird 1956), sugar accumulation could occur as a type of salt-accumulation process. If this were so, sucrose (or glucose) would presumably be converted to an ionic form such as the sucrosyl phosphate ion. This would require energy in the form of energy-rich phosphate bonds ($\sim$ P). Further energy would be required to provide for the accumulation of this ion against a concentration gradient. If it is assumed that the respiration increment which occurred when sucrose and glucose were added to the slices provided the energy for the accumulation of those sugars, then 17.8 μ g sucrose was respired to provide for the accumulation of 148.0 μ g sucrose, and 26.2 μ g glucose was respired to provide for the accumulation of 189.0 μ g glucose (from Table 3). The energy yield

of aerobic respiration is approximately 38 $\sim$ P per hexose molecule completely respired. Hence, 9 $\sim$ P were generated per sucrose molecule accumulated, and 5 $\sim$ P per glucose molecule accumulated. If glucose was first converted to sucrose before being accumulated (as suggested by data in Figure 9 and Table 2), requiring 1 $\sim$ P per sucrose molecule (Shukla and Prabhu 1959), there would again be 9 $\sim$ P available per sucrose molecule. This, for example, would be sufficient to permit phosphorylation of the sucrose molecule (requiring 1-2 $\sim$ P) and still provide for its subsequent accumulation at an efficiency of about 20 per cent. assuming the accumulation mechanism proposed by Lundegårdh (1945) and Robertson and Wilkins (1948).

In the field, during active photosynthesis, the sugar concentration of the leaf is less than 2-3 per cent. of the fresh weight, 70 per cent. of the sugar being sucrose (Hartt 1935); but in the young and immature internodes the concentration is 4-10 per cent. (75-85 per cent. as sucrose), and in the old mature internodes at the base of the stem the concentration may be as high as 20 per cent. (95 per cent. as sucrose) (Lal and Srivastava 1945; Hes 1949). In the mature internodes the sugar concentration is lowest (*c.* 12 per cent.) in the season when cane growth is at a maximum, and rises to a peak in the "ripening" season (Das 1936); these two periods being in summer and in late autumn-early winter respectively in Australia (Lowndes 1956). At least a part of the sugar stored in the mature internodes during ripening comes directly from current photosynthesis in the leaves (Hartt and Burr (1953). *In vitro*, the sugar accumulation rate shown by a particular tissue slice sample was closely related to the metabolic state of the parent tissue in the field. The maximum accumulation rate, *c.* 6.0 mg/g tissue/day, occurred in slices of tissue taken from internodes in which cell division and enlargement had just ceased, and which were starting to store sugars rapidly as part of the maturation process. At this time in the field, the endogenous sugar concentration rises from 3 to 11 per cent. in 2-4 weeks (2.8-5.6 mg/g tissue/day). In older internodes where the storage was slower, the rate of sugar accumulation in tissue slices was correspondingly less (Table 6). In fully mature tissues (sugar concentration of tissue over 16 per cent., June-August), there was no detectable accumulation. The decreased uptake was not a function of the increased concentration gradient between solution and tissue, since increasing the solution concentration to 25 per cent. did not increase the rate of uptake. Thus the laboratory behaviour of the tissue slices is closely comparable to the field behaviour of the intact tissue, suggesting that it might be possible to use laboratory experiments to screen new cane varieties for their ability to store sugar.

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