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Sugar Accumulation in Sugarcane

CARRIER-MEDIATED ACTIVE TRANSPORT OF GLUCOSE

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ABSTRACT

The rate-limiting reaction for glucose uptake in storage tissue of sugarcane, *Saccharum officinarum* L., appears to be the movement of glucose across the boundary between the free space and the metabolic compartments. The mechanism for uptake of glucose across this boundary has been studied using 3-O-methyl glucose, an analogue of glucose which is not metabolized by sugar-cane tissue.

This analogue is taken up by sugarcane storage tissue at a similar rate to glucose. Its rate of uptake follows Michaelis-Menten kinetics, K_m = 1.9 mM, and it is competitively inhibited by glucose, K_i = 2 to 3 mM. Glucose uptake is similarly inhibited by 3-O-methyl glucose. Uptake of 3-O-methyl glucose is energy-dependent and does not appear to be the result of counterflow of glucose.

It is concluded that glucose and 3-O-methyl glucose uptake across the boundary between the free space and the metabolic compartment in this tissue is mediated by an energy-dependent carrier system capable of accumulating the sugars against a concentration gradient.

Sucrose entering the storage parenchyma of sugarcane from the vascular tissue is hydrolyzed by invertases in the free space (20). Subsequent movement from the free space into the inner space of these cells is as hexoses (9, 20). Within the inner space hexoses are rapidly reconverted to sucrose by a sequence of reactions involving hexokinase, glucosephosphate isomerase, phosphoglucomutase, UDP-glucose pyrophosphorylase (9), UDP-glucose-fructose-6-phosphate transglucosylase (8), and a specific sucrose phosphate phosphohydrolase (12). The sucrose so formed appears in the vacuole.

In studies on the uptake of ^{14}C-labeled glucose, Michaelis-Menten kinetics were obtained, but with a 20-fold variation in the estimated K_m value (3, 5, 11). Initially, almost all of the radioactivity stored in the tissue was in sucrose (5). Hitherto it has not been possible to decide whether the rate-limiting step was carrier-mediated transport of glucose into the tissue, as proposed by Bieleski (3), or one of the sequence of enzymic reactions leading to synthesis of sucrose.

In this paper we describe experiments using 3-O-methyl glucose, an analogue of glucose which is not metabolized by sugarcane. We have obtained new evidence consistent with the existence of a specific carrier for glucose and 3-O-methyl glucose which mediates movement of these sugars into the metabolic compartment. The carrier system appears to be both energy-dependent and capable of accumulation of the sugars against a concentration gradient.

MATERIALS AND METHODS

Selection of Tissue. Tissue was selected from the rapidly expanding zone from the young internodes of field-grown sugarcane, *Saccharum officinarum* L. var. Pindar. Standard discs 7 mm diameter × 1 mm were cut from the storage tissue of this zone using a sharp cork borer and microtome blade, and washed for 1 hr in running tap water to remove broken cell contents.

Measurement of Sugar Uptake. Discs (0.7 g) were incubated in 2 ml of aqueous medium in 10-ml flasks shaken at 140 cpm, preliminary experiments having shown that maximum rates of uptake occur under these conditions. Incubation was at 30 C unless otherwise stated.

For measurement of uptake of glucose or 3-O-methyl glucose, discs were incubated in solutions of the sugars containing ^{14}C-glucose or 3-O-methyl ^{14}C-glucose (0.005–0.5 μc per μmole) and harvested after the required intervals by washing the tissue for 1 hr in running tap water to remove free-space sugar, then extracting with 2 ml of 95% ethanol for 24 hr. Aliquots of up to 0.5 ml of the ethanolic extracts were counted by liquid scintillation in 5 ml of toluene (containing 0.4% PPO, 0.01% POPOP):Triton-X-100:water 50:25:9 (v/v/v) (17). This mixture counted aqueous and ethanolic extracts with the same efficiency. Measurements of leakage rates of sugars from previously labeled tissue slices into aqueous media were made by counting ^{14}C appearing in the medium using the same scintillation mixture.

Crude Enzyme Preparations. Extract 1. Storage tissue (2 g) from the rapidly expanding zone of young internodes (outer rind removed) was ground with sand in 5 ml of 0.5 M sodium phosphate buffer pH 8.0, 20 mM sodium dithionite at 2 C in a prechilled mortar, and the mixture was squeezed through muslin and dialyzed for 24 hr against water at 2 C. This enzyme preparation was used for assays of hexokinase activity.

Extract 2. Storage tissue (60 g) was chilled in iced water, ground at 2 C in 80 ml of 0.5 M sodium phosphate, pH 7.5, containing 10 mM EDTA and 5 mM dithiothreitol, and squeezed through muslin and centrifuged at 5000*g* for 10 min. Solid ammonium sulfate was added to 70% saturation, the precipitate was collected by centrifugation, redissolved in 5 ml of 5 mM sodium phosphate, pH 7.5, 1 mM EDTA, and passed at 2 C through a column of Sephadex G-25 equilibrated with the same buffer. This enzyme preparation was used for assays of phosphoglucomutase and glucosephosphate isomerase.

ASSAYS ON EXTRACTED ENZYMES

Hexokinase. Reaction mixtures contained 0.1 M sodium phosphate, pH 8.0, 10 mM ATP, 10 mM $MgCl_2$, 10 mM ammonium molybdate, 18 μM ^{14}C-glucose (110 mc/mmole) and crude enzyme extract 1 and were incubated at 30 C. Samples

were taken over a time sequence, dried onto Whatman No. 1 paper, and hexoses were separated from hexose phosphates by chromatography in ethyl acetate:pyridine:water (8:2:1, v/v). Hexoses and hexosephosphates were counted by end-window G.M. counting.

Glucosephosphate Isomerase. Reaction mixtures contained 80 mM sodium phosphate, pH 7.5, 2 mM $MgCl_2$, 0.37 mM NADP, 5 mM fructose-6-phosphate, Sigma glucose-6-phosphate dehydrogenase Type-X, and crude enzyme extract 2. Reaction was followed at 22 C by measuring $\Delta A^{1\text{ cm}}_{340\text{ nm}}$.

Phosphoglucomutase. Reaction mixtures contained 50 mM Tricine-HCl buffer, pH 7.5, 2 mM $MgCl_2$, 0.1 mM NADP, 5 mM glucose-1-phosphate, Sigma glucose-6-phosphate dehydrogenase Type-X and crude enzyme extract 2. Reaction was followed at 22 C by measuring $\Delta A^{1\text{ cm}}_{340\text{ nm}}$.

ASSAYS ON ENZYME ACTIVITIES IN INTACT TISSUE SLICES

Respiration. Respiration was measured by rapidly stirring 22 discs (7 mm × 1 mm) of tissue in a closed cell containing 20 ml of water at 30 C and following the rate of oxygen uptake from the system using a Clark oxygen electrode.

Outer Space Invertase. Standard discs (7 mm × 1 mm) were cut, washed, and incubated under the above standard conditions in 23 mM ^{14}C-sucrose (0.034 μc/μmole), 8 mM sodium acetate, pH 5.5, and $CaCl_2$ 2 μg/ml. After 4 hr, 10 μl of medium was dried onto Whatman No. 1 paper for subsequent chromatography in ethyl acetate:pyridine:water (8:2:1, v/v) to separate glucose, fructose, and sucrose, and the extent of hydrolysis of sucrose in the medium was determined by end-window G.M. counting. The tissue discs (after 4 hr) were washed in running water for 1 hr to remove free-space sugars, then extracted for 24 hr with 2 ml of 95% ethanol, and the ethanolic extract was counted by liquid scintillation to determine the amount of sugar taken up by the tissue. Outer space invertase was calculated by summing the hexoses in the medium and total sugar uptake by the tissue (20).

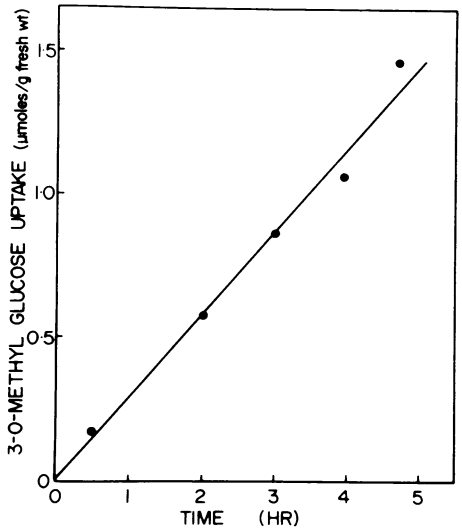

FIG. 1. Uptake of 3-O-methyl glucose into nonfree space. Tissue discs were cut, washed 1 hr, and incubated under standard conditions in 5 mM 3-O-methyl ^{14}C-glucose (6 μc/μmole). At the times shown, tissue discs were washed in running water for 1 hr to remove free space sugars, extracted with ethanol, and aliquots of the extract were counted by liquid scintillation to determine sugar uptake.

Table I. *Rate of Uptake of Sugars by Tissue Discs*

Tissue discs were cut, washed, and incubated under standard conditions for 3 hr in either 10 mM ^{14}C-glucose (0.026 μc/μmole) or 10 mM 3-O-methyl ^{14}C-glucose (0.006 μc/μmole), then washed 1 hr in running water, extracted with 95% ethanol for 24 hr, and the ethanolic extract was counted by liquid scintillation.

	Uptake	SE[1]
	μmoles hr^{-1} g^{-1} fresh wt	
Glucose	0.80	± 0.023
3-O-Methyl glucose	0.72	± 0.047

[1] Two SE mean.

Reactions Involved in Sucrose Resynthesis. Tissue discs were cut, washed, and incubated under standard conditions in 0.1 mM ^{14}C-sucrose (10 μc/μmole), 5 mM sodium acetate, pH 5.5, and $CaCl_2$ 2 μg/ml. After 3.5 hr the tissue was washed for 1 hr in running water and extracted overnight in 2 ml of 95% ethanol. Samples both from the medium and from this ethanol extract were chromatographed in ethyl acetate:pyridine:water (8:2:1, v/v), and the distribution of radioactivity in hexose phosphates, sucrose, glucose, and fructose was determined.

Chemicals. ^{14}C-Sucrose and ^{14}C-glucose were obtained from the Radiochemical Centre, Amersham and 3-O-methyl ^{14}C-glucose was the product of New England Nuclear Corporation.

RESULTS

Linearity of Uptake of 3-O-Methyl Glucose. When washed discs of sugarcane storage tissue were incubated in 5 mM 3-O-methyl glucose, this sugar was taken up into nonfree space at a linear rate over at least 5 hr (Fig. 1). For kinetic studies, incubation periods of up to 4 hr preceded and followed by washes of 1 hr in running water were used to measure "initial rates" of uptake. Since the absolute rate of uptake of sugars varied with the physiological age of the tissue, it was necessary to include controls in many experiments as a basis of reference.

Uptake of 3-O-Methyl Glucose Compared with Uptake of Glucose. Tissue discs were incubated in either 10 mM ^{14}C-glucose or 10 mM 3-O-methyl ^{14}C-glucose for several hours. As shown in Table I, 3-O-methyl glucose and glucose were taken up by the tissue at similar rates.

The sugars accumulated by tissues from these two solutions were separated by chromatography. Radioactivity taken up from ^{14}C-glucose solution was distributed 79% in sucrose, 7% in glucose, 4% in fructose, and 10% in sugar phosphates as expected in juvenile cane storage tissue. The tissue discs incubated in 3-O-methyl ^{14}C-glucose were extracted both with 95% ethanol and with chloroform:methanol (2:1, v/v). These extracts were subjected to chromatography in butanol:acetic acid:water (5:1:4, v/v, upper phase) and in ethyl acetate:pyridine:water (8:2:1, v/v). After removal of the solvents, aqueous solutions of the extracts were also passed through columns of Dowex 1 × 8-Cl^- and Dowex 50-H^+ which were subsequently eluted with 4 N formic acid and 1 N NH_4OH respectively. All detectable radioactivity (99.95%) taken up from 3-O-methyl ^{14}C-glucose solution remained as 3-O-methyl glucose inside the tissue.

A crude extract of sugarcane hexokinase failed *in vitro* to phosphorylate 3-O-methyl glucose.

Uptake of 3-O-Methyl Glucose against a Gradient. Demonstration that 3-O-methyl glucose could be taken up against a concentration gradient was achieved by increasing the period of incubation of the tissue (Table II). Sufficient 3-O-methyl glucose was taken up by the tissue from the external solution in 17 hr to bring the tissue slices to an internal concentration not less than 2.5 times the concentration in the external solu-

Table II. *Uptake of 3-O-Methyl Glucose Against a Gradient*

Tissue discs (0.7 g) were cut, washed, and incubated under standard conditions in 5 mM 3-O-methyl ^{14}C-glucose (2.2 μc/mmole) for 17 or 23 hr. The medium was replaced every 2 hr. 3-O-Methyl glucose uptake was measured after washing out free-space sugars as described under Figure 1. Calculations: A: assuming 3-O-methyl glucose is in the total volume of tissue fluid, 0.5 ml/0.7 g tissue discs; B: assuming 3-O-methyl glucose is only in cytoplasmic volume, 0.02 ml/0.7 g tissue discs. External concentration 3-O-methyl glucose was 5 mM in all cases.

Experiment No.	Incubation Time	Uptake 3-O-Methyl Glucose	Internal Concn 3-O-Methyl Glucose (calculated)	
			A	B
	hr	*μmoles/0.7 g*	*mM*	*M*
1	17	7.1	13	0.3
	23	8.8	16	0.4
2	17	6.1 ± 1.7[1]	12	0.3

[1] Two SE mean.

tion. In estimating the internal concentration of 3-O-methyl glucose in these experiments, it was assumed that the 3-O-methyl glucose occupied the entire volume of the 7 mm × 1 mm discs. If 3-O-methyl glucose was taken up only into the cytoplasm (2.5% of the tissue volume, Hawker [10]), the gradient would be more than 70-fold and have an internal concentration of 0.3 to 0.4 M and an external concentration of 5 mM.

By estimating the bacterial population present on the tissue discs at the end of these experiments, it was also shown that bacteria were not responsible for the uptake observed. After 17 hr of incubation in 5 mM 3-O-methyl glucose, 0.7 g storage tissue was washed in running water for 1 hr then ground in sterile water, the mixture serially diluted and plated out onto glucose-agar media. Bacterial cells (125,000) of spherical diameter 2 μ were detected in 0.7 g of tissue; a total bacterial volume of 5×10^{-7} ml. For bacteria alone to be responsible for the uptake of 3-O-methyl glucose measured, the minimum internal concentration of 3-O-methyl glucose would be 1.2×10^{4} M.

Kinetics of 3-O-Methyl Glucose Uptake: Competitive Inhibition by Glucose. The rate of uptake of 3-O-methyl glucose by tissue slices was dependent on the concentration of the

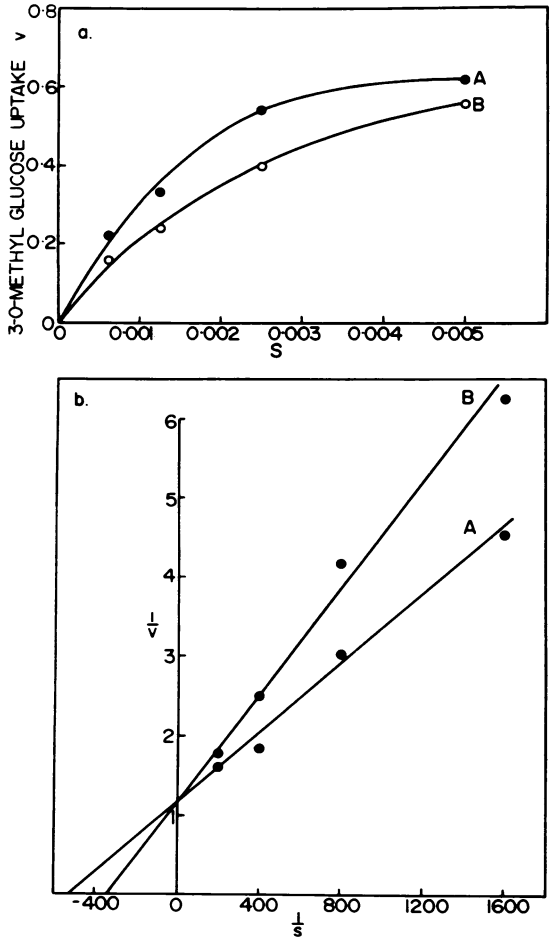

FIG. 2. Competitive inhibition of 3-O-methyl glucose uptake by glucose. Tissue discs were cut, washed and incubated under standard conditions in 3-O-methyl ^{14}C-glucose at the concentrations shown, A in the absence and B in the presence of 1 mM glucose. After 4 hr, all tissue was washed, and 3-O-methyl glucose uptake was determined as in Figure 1. a: Plot of v = rate of uptake of 3-O-methyl glucose (μmoles hr^{-1} g^{-1} fresh wt) against S = concentration (M) of 3-O-methyl glucose in the medium. b: Lineweaver-Burk plot (14) of data from a.

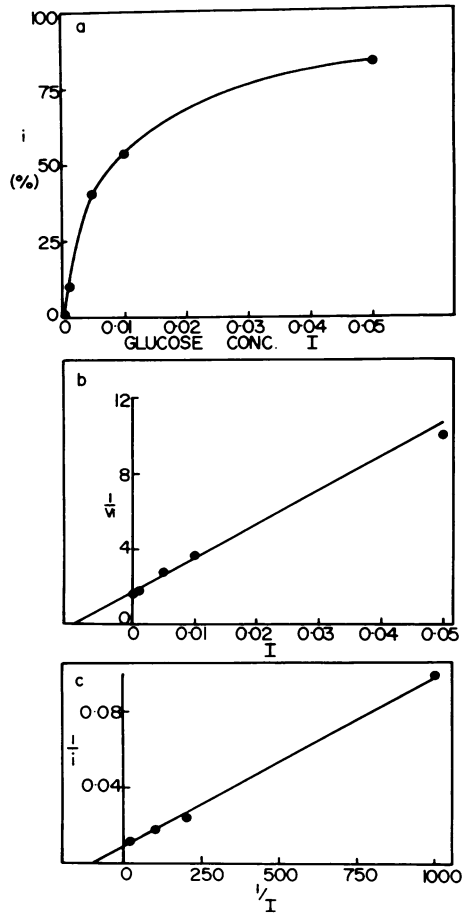

FIG. 3. Inhibition of 3-O-methyl glucose uptake by glucose. Tissue discs were cut, washed, and incubated under standard conditions in 4 mM 3-O-methyl ^{14}C-glucose (0.01 μc/μmole) in the presence of the range of glucose concentrations shown. After 4 hr, all tissue was washed, and 3-O-methyl glucose uptake was determined as in Figure 1. a: Plot of i = inhibition of 3-O-methyl glucose uptake (%) against I = concentration (M) of glucose in the medium. b: Plot of 1/vi against I (Webb, 23) where vi = 3-O-methyl glucose uptake, μmoles hr^{-1} g^{-1} fresh wt. c: Plot of 1/i against 1/I (Dixon, 4).

Table III. *Relative Activities of Some Enzymes and Cellular Processes in the Presence of 3-O-Methyl Glucose*

Respiratory rates and enzyme activities are quoted in μmoles substrate taken up or converted per g fresh wt·hr. Sucrose uptake is the relative activity after 3.5 hr of accumulation from 0.1 mM ^{14}C-sucrose. The internal distribution of the label was determined on ethanolic extracts after separation by paper chromatography. For hexokinase assay, 3-O-methyl glucose was present at a concentration of 1.3 mM; for other measurements it was at 20 mM.

System	Control	3-O-Methyl Glucose
Hexokinase	1.44	1.46
Glucosephosphate isomerase	2.03	2.10
Phosphoglucomutase	0.036	0.036
Outer space invertase	1.97	2.14
Respiration	1.03	1.00
Sucrose uptake	1.00	0.77
Internal distribution of label from ^{14}C-sucrose uptake as percentage of total		
Sucrose	65	63
Glucose	12	13
Fructose	10	11
Hexosephosphates	13	13

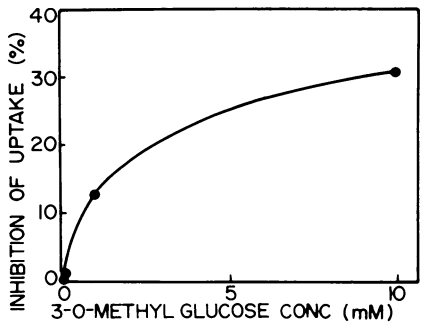

FIG. 4. Inhibition of glucose uptake by 3-O-methyl glucose. Tissue discs were cut, washed, and incubated under standard conditions in 10 mM ^{14}C-glucose (0.03 μc/μmole) in the presence of the range of 3-O-methyl glucose concentrations shown. After 4 hr, all tissue was washed, and glucose uptake was determined as in Figure 1. Inhibition of glucose uptake (1%) is plotted against concentration (mM) of 3-O-methyl glucose in the medium.

sugar in the external medium. As shown in Figure 2, rate of uptake was related to the external sugar concentrations by Michaelis-Menten kinetics. K*m* for 3-O-methyl glucose uptake in this system was 1.9 mM. Uptake of 3-O-methyl glucose by tissue discs was inhibited by glucose. The kinetics of this inhibition (Fig. 2) indicate that glucose is a competitive inhibitor of 3-O-methyl glucose uptake, having a dissociation constant K*i* = 2 to 3 mM. The inhibition of 3-O-methyl glucose uptake by glucose reached at least 85% (Fig. 3). Both the plot of Dixon (4) and the double reciprocal plot of Webb (23) give linear relationships (Fig. 3, b and c) and confirm the K*i* value 2 to 3 mM for glucose inhibition.

Data for 3-O-methyl glucose inhibition of glucose uptake are shown in Figure 4. There was approximately 30% inhibition of glucose uptake from 10 mM glucose by 10 mM 3-O-methyl glucose, and approximately 30% inhibition of 3-O-methyl glucose uptake from 4 mM 3-O-methyl glucose by 4 mM glucose (Fig. 3).

Effects of 3-O-Methyl Glucose on Reactions Leading to Sucrose Synthesis. Label from ^{14}C-glucose in the medium initially appears in the tissue primarily in sucrose, and subsequently hexoses are formed from hydrolysis of stored sucrose (5). Before any conclusions could be drawn from these studies about a common carrier mechanism for 3-O-methyl glucose and glucose uptake, it had to be shown that 3-O-methyl glucose inhibition of glucose uptake was not mediated by interference with the metabolic sequence leading to sucrose synthesis. We measured the effects of 3-O-methyl glucose on the *in vitro* activities of hexokinase, glucosephosphate iomerase, and phosphoglucomutase in crude extracts of sugarcane storage tissue. We also examined the effects of 3-O-methyl glucose on the respiration and free space invertase activity of tissue discs and on the pattern of distribution of radioactivity taken up from ^{14}C-sucrose solutions into the inner space of tissue discs. These results are summarized in Table III.

At concentrations causing substantial inhibition of hexose uptake, 3-O-methyl glucose did not inhibit either free space invertase, hexokinase, glucosephosphate isomerase, phosphoglucomutase, or respiration. 3-O-Methyl glucose inhibited 23% of the total uptake of sugar from ^{14}C-sucrose by tissue discs, which is expected since sucrose is hydrolyzed prior to uptake. However, the internal distribution between individual sugars and sugar phosphates of the radioactivity taken up from ^{14}C-sucrose into these tissue discs was the same in the presence and absence of 3-O-methyl glucose. There was, therefore, no effect of this compound on the rest of the enzyme systems involved in the resynthesis and breakdown of stored sucrose in this tissue.

Table IV. *Inhibition of Fructose Uptake by 3-O-Methyl Glucose*

Tissue slices were cut, washed and incubated under standard conditions in 10 mM ^{14}C-glucose or 10 mM ^{14}C-fructose, with or without 20 mM 3-O-methyl glucose. After 4 hr tissue was washed and extracted to determine uptake by the standard procedure.

Sugar in External Medium	Uptake				Inhibition of Uptake
	− 3-O-Methyl glucose	2 SE	+ 3-O-Methyl glucose	SE[1]	
	μmoles/g fresh wt·hr		μmoles/g fresh wt·hr		%
^{14}C-Glucose	0.84	± 0.12	0.58	± 0.12	31
^{14}C-Fructose	0.61	± 0.09	0.50	± 0.02	17

[1] Two SE mean.

Table V. *Effects of Low Temperature and Low Oxygen on 3-O-Methyl Glucose Uptake*

Tissue discs were cut, washed, and incubated with normal shaking in 2 ml 5 mM 3-O-methyl ^{14}C-glucose (7 μc/mmole) in either (*a*) sealed 10-ml flasks equilibrated with nitrogen by bubbling for several minutes and at 30 C or (*b*) unsealed flasks equilibrated with air and at either 1 C or 30 C. After 4 hr, tissues were washed to remove free-space sugars and harvested for uptake determination by the standard procedure.

Treatment		Uptake of 3-O-Methyl glucose	SE[1]
Atmosphere	Temperature		
		μmoles/g·hr	
Air	30 C	1.12	± 0.14
Nitrogen	30 C	0.36	± 0.06
Air	1 C	0.15	± 0.004

[1] Two SE mean.

The only effect of 3-O-methyl glucose detected in sugarcane storage tissue was inhibition of the uptake of glucose (Fig. 4), and to a lesser extent uptake of fructose (Table IV), without concurrent change in internal or external sugar metabolism and without metabolism of 3-O-methyl glucose itself.

These results show that the 3-O-methyl glucose effect on glucose uptake is not at one of the sequence of enzymatic steps leading to sucrose synthesis. They are consistent with a common carrier hypothesis for glucose and 3-O-methyl glucose transport into the tissue.

Effect of Low Temperature and Low Oxygen on 3-O-Methyl Glucose Uptake. When tissue discs were incubated in the absence of oxygen or at low temperature (Table V) uptake of 3-O-methyl glucose was inhibited.

These results are expected if 3-O-methyl glucose influx was via an energy-linked carrier system. However, they could also be generated by counterflow, that is, uphill transport of one substrate mediated by downhill transport of another, both competing for the same carrier (18).

If one postulates a carrier that equilibrates rapidly with substrates at the inner and outer surfaces, decreased initial influx of 3-O-methyl glucose would be expected if the endogenous glucose concentration were decreased by the nitrogen and low temperature treatments. The experiment in air and nitrogen was repeated measuring both uptake of 3-O-methyl glucose and the efflux of hexoses. It was found that the efflux of endogenous hexose increased in N_2 while influx of 3-O-methyl glucose decreased (Table VI). This is not consistent with the postulate.

Alternatively, counterflow could be mediated by a carrier which does not equilibrate rapidly with substrates at the inner and outer surfaces. In this case a rise in endogenous glucose could tie up significant amounts of carrier, making less available for mediating influx of 3-O-methyl glucose. This postulate would accommodate the results of Tables V and VI, but if true would require that it be possible to do the experiment in reverse. The reverse experiment is to preload the tissue with 3-O-methyl glucose, then inhibit efflux by addition of glucose to the external medium. The result of such an experiment is shown in Figure 5, there being no effect of exogenous glucose on the efflux rate of 3-O-methyl glucose.

It would appear the transport system is an energy-coupled carrier, that is not counterflow-dependent.

Effect of Sodium Ions on 3-O-Methyl Glucose Uptake. In order to test for the Na^+ activated transport system demonstrated in animal tissues (7), tissue discs were maintained in 3-O-methyl glucose medium with and without 1 mM NaCl for 17 hr with frequent changes of the medium. Despite this extended washing of the tissue without Na^+, no effect was observed on the rate of 3-O-methyl glucose uptake (Table VII).

Table VI. *Concurrent 3-O-Methyl Glucose Uptake and Glucose Leakage*

Tissue discs were cut, washed, and incubated in 5 mM 3-O-methyl ^{14}C-glucose (0.01 μc/μmole) with normal shaking either in unsealed flasks equilibrating with normal air or in sealed flasks equilibrated with nitrogen by bubbling for several minutes. After 4 hr, reducing sugars in the medium were estimated (15) after chromatography to remove 3-O-methyl glucose. The tissue discs were washed 1 hr to remove free-space sugars, then 3-O-methyl glucose uptake was determined by extraction and counting by the standard procedure.

Treatment	Uptake of 3-O-Methyl Glucose	SE[1]	Leakage of Glucose and Fructose	2 SE
	μmoles/g·hr		*μmoles/g·hr*	
Air 30 C	1.15	± 0.13	3.7	± 0.6
Nitrogen 30 C	0.24	± 0.17	5.6	± 1.2

[1] Two SE mean.

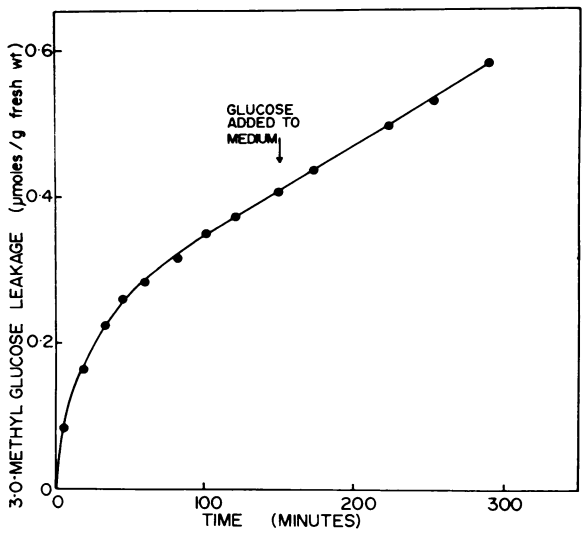

FIG. 5. Effect of the addition of glucose externally on the leakage rate of 3-O-methyl glucose. Tissue discs were cut, washed, and incubated under standard conditions in 5 mM 3-O-methyl ^{14}C-glucose (0.24 μc/μmole) for 17 hr at 30 C, changing the medium every 2 to 3 hr, then washed 45 min in running water. This figure shows the rate of leakage of 3-O-methyl glucose (μmoles g^{-1} fresh wt) into the external solution when incubation was continued in 3 ml water at 1 C with normal shaking (0.2-ml samples of this medium were removed at the time intervals shown and counted to determine the rate of leakage of 3-O-methyl glucose). Glucose was added to the medium after 150 min to a final concentration of 5 mM. Incubation at 1 C was used to prevent uptake of leaked 3-O-methyl glucose.

Table VII. *Effect of Sodium Ions on 3-O-Methyl Glucose Uptak*

Tissue discs were cut, washed for 1 hr, and incubated under standard conditions in either 5 mM 3-O-methyl ^{14}C-glucose alone or 5 mM 3-O-methyl ^{14}C-glucose + 1 mM NaCl. Solutions were changed every 2 to 3 hr. After 17 hr, tissue was washed to remove free space sugar and extracted for uptake measurements by the standard procedure.

Additions to Medium	Uptake of 3-O-Methyl Glusose
	μmoles/hr·g fresh wt
Nil	8.7
NaCl (1 mM)	8.6

DISCUSSION

The results presented show that glucose uptake into cane parenchyma cells is limited by a carrier-mediated step, and not by one of the enzymes concerned with sucrose synthesis. The same carrier transports 3-O-methyl glucose, which accumulates in the tissue unchanged. The carrier-mediated step is energy-linked, will mediate accumulation against a concentration gradient, but does not have the characteristics of counterflow.

3-O-Methyl glucose has been used previously as a glucose analogue to study sugar transport in animal systems (16, 18), in bacteria (13), and in plants (19). In *Escherichia coli* it was phosphorylated (13), but in carrot tissues no products of metabolism were detected (19).

A number of mechanisms have been proposed for glucose

uptake in other tissues which involve formation of metabolic intermediates. These include phosphorylation, either with subsequent hydrolysis as occurs during accumulation of sucrose in sugarcane (8, 20), or without subsequent hydrolysis as in the case of glucose transport in *E. coli* by the phosphoenolpyruvate-phosphotransferase system (13). Behrens and Leloir (2) proposed that glucose is taken up by a mechanism which involves the formation of UDP-glucose, and subsequently a glucosylated lipid intermediate. Despite the similar rates of uptake of 3-O-methyl glucose and glucose by tissues no derivatives of 3-O-methyl ^{14}C-glucose were found in either aqueous ethanolic extracts or chloroform-methanol extracts of sugarcane storage tissue. Sacktor (21, 22) proposed a mechanism involving the formation of trehalose-phosphate and trehalose as intermediates in glucose transport in insect gut and in animal kidneys and intestine. Following the discovery of trehalase activity in sugarcane storage tissue (6), we have searched particularly for trehalose among the sugars which occur in trace amounts in cane but without success. We conclude therefore that the major pathway of glucose uptake into sugarcane tissue does not involve these mechanisms. Another proposed mechanism involves allosteric transitions of a carrier moving glucose by coupling to a Na^+-pump system (7). We could find no evidence for such a system in sugarcane.

A mechanism we have not been able to exclude would be analogous to the β-galactoside transport system in *E. coli* which is coupled for energy supply to a lactic dehydrogenase system (1).

LITERATURE CITED

1. Barnes, E. M., Jr. and H. R. Kaback. 1970. β-Galactoside transport in bacterial membrane preparations: energy coupling via membrane-bound D-lactic dehydrogenase. Proc. Nat. Acad. Sci. U.S.A. 66: 1190–1198.
2. Behrens, N. H. and L. F. Leloir. 1970. Dolichol monophosphate glucose: an intermediate in glucose transfer in liver. Proc. Nat. Acad. Sci. U.S.A. 66: 153–159.
3. Bieleski, R. L. 1962. The physiology of sugarcane. V. Kinetics of sugar accumulation. Aust. J. Biol. Sci. 15: 429–444.
4. Dixon, M. 1953. The determination of enzyme inhibitor constants. Biochem. J. 55: 170–171.
5. Glasziou, K. T. 1961. Accumulation and transformation of sugars in stalks of sugarcane. Origin of gluocse and fructose in the inner space. Plant Physiol. 36: 175–179.
6. Glasziou, K. T. and K. R. Gayler. 1969. Sugar transport: occurrence of trehalase activity in sugarcane. Planta 85: 299–302.
7. Goldner, A. M., S. G. Schultz, and P. F. Curran. 1969. Sodium and sugar fluxes across the mucosal border of rabbit ileum. J. Gen. Physiol. 53: 362–383.
8. Hatch, M. D. 1964. Sugar accumulation by sugarcane storage tissue. The role of sucrose phosphate. Biochem. J. 93: 521–526.
9. Hatch, M. D., J. A. Sacher, and K. T. Glasziou. 1963. Sugar accumulation cycle in sugarcane. I. Studies on enzymes of the cycle. Plant Physiol. 38: 338–343.
10. Hawker, J. S. 1965. The sugar content of cell walls and intercellular spaces in sugarcane stems and its relation to sugar transport. Aust. J. Biol. Sci. 18: 959–969.
11. Hawker, J. S. and M. D. Hatch. 1965. Mechanism of sugar storage by mature stem tissue of sugarcane. Physiol. Plant 18: 444–453.
12. Hawker, J. S. and M. D. Hatch. 1966. A specific sucrose phosphatase from plant tissues. Biochem. J. 99: 102–107.
13. Kaback, H. R. 1968. The role of the phosphoenolpyruvatephosphotransferase system in the transport of sugars by isolated membrane preparations of *Escherichia coli*. J. Biol. Chem. 243: 3711–3724.
14. Lineweaver, H. and D. Burk. 1934. The determination of enzyme dissociation constants. J. Amer. Chem. Soc. 56: 658–666.
15. Park, J. T. and M. J. Johnson. 1949. A submicro-determination of glucose. J. Biol. Chem. 181: 149–151.
16. Park, C. R., D. Reinwein, M. J. Henderson, E. Cadenas, and H. E. Morgan. 1959. The action of insulin on the transport of glucose through the cell membrane. Amer. J. Med. 26: 647–684.
17. Patterson, M. S. and R. C. Greene. 1965. Measurement of low energy beta-emitters in aqueous solution by liquid scintillation counting of emulsions. Anal. Chem. 37: 854–857.
18. Regen, D. M. and H. E. Morgan. 1964. Studies of the glucose-transport system in the rabbit erythrocyte. Biochim. Biophys. Acta 79: 151–166.
19. Reinhold, L. and Z. Eshhar. 1968. Transport of 3-O-methyl glucose into and out of storage cells of *Daucus carota*. Plant Physiol. 43: 1023–1030.
20. Sacher, J. A., M. D. Hatch, and K. T. Glasziou. 1963. Sugar accumulation cycle in sugarcane. III. Physical and metabolic aspects of cycle in immature storage tissues. Plant Physiol. 38: 348–354.
21. Sacktor, B. 1965. Energetics and respiratory metabolism of muscular contraction. *In:* M. Rockstein, ed., Physiology of Insecta, Vol. 2. Academic Press, New York. pp. 483–582.
22. Sacktor, B. 1968. Trehalase and the transport of glucose in the mammalian kidney and intestine. Proc. Nat. Acad. Sci. U.S.A. 60: 1007–1014.
23. Webb, J. L. 1963. Enzyme and Metabolic Inhibitors, Vol. 1. Academic Press, New York.