Plant Physiol. (1984) 74, 16–20
0032-0889/84/74/0016/05/$01.00/0

Sugar Uptake by Cotton Tissues

LEAF DISC *VERSUS* CULTURED ROOTS

Received for publication March 29, 1983 and in revised form September 2, 1983

DONALD L. HENDRIX
Western Cotton Research Laboratory, 4135 East Broadway Road, Phoenix, Arizona 85040

ABSTRACT

The tissue accumulation of sucrose, glucose, and fructose has been studied in cultured cotton (*Gossypium hirsutum* L.) roots and leaf discs. Sucrose uptake by both tissues from high apoplastic concentrations was independent of pH but has a slightly acidic pH optimum from low concentrations. Like other higher plant tissues, cotton root cells accumulate sucrose via a 'saturable,' inhibitor-sensitive mechanism and a linear, inhibitor-resistant mechanism. The linear mechanism of sucrose uptake is not as pronounced in leaf disc data as it is in root data. Further, sucrose uptake by cotton leaf discs is more resistant than uptake by root cells to pH alterations, inhibitors, and monosaccharides in the uptake medium. The saturable phase of sucrose influx into cotton root is eliminated by glucose, fructose, and high pH. Sucrose influx into both tissues is not altered by osmotica up to 200 milliOsmolar. Sucrose accumulated by both tissues is rapidly converted to other chemical forms, especially in root tissue where only approximately 50% remains as neutral sugars 1 hour following the start of radiolabel exposure. Although the entry of radiolabeled sucrose is faster in abraded leaf discs, they give the same response patterns to pH, inhibitors, and monosaccharide as do unabraded discs.

The sucrose accumulation kinetics of cotton roots and leaf discs differ. These differences may be related to the physiological roles (source *versus* sink) of the two tissues in the intact plant.

Cell accumulation of sugar in cells from the apoplastic compartment is of major importance to short distance carbohydrate movement in plant tissues (5, 6). Sucrose accumulation in tissues isolated from several plant species seems to consist of a saturable component, most apparent at low sucrose levels, plus a nonsaturable component (17, 18, 21). Evidence for this interpretation of these kinetics stems from, among other things, the elimination of the saturable, but not the nonsaturable, component of sucrose uptake by inhibitors, particularly the organic mercurials. The specificity of the mercurials in blocking sucrose accumulation has been demonstrated by Giaquinta (6, 7) who found they caused a sharp decrease in sucrose influx but did not effect (at the concentrations employed) *Beta* leaf photosynthesis, sucrose efflux, respiration, or hexose uptake.

The primary translocation product in cotton is thought to be sucrose (11). The hydrolysis products of sucrose, fructose, and glucose, are also known to play key roles in carbohydrate movement in those plant species which employ sucrose hydrolysis between assimilate source and sink (4, 8, 9). Hampson *et al.* (11, 12) analyzed carbohydrate accumulation by excised cotton hypocotyl and concluded that cellular invertase is functionally absent in this tissue. However, preliminary experiments (D. Hendrix and D. Doman, unpublished) showed that asymmetrically labeled sucrose is inverted during accumulation by isolated cotton embryos. The uptake of glucose and fructose and the effect of these sugars upon sucrose influx in cotton root and leaf discs was therefore investigated.

Since it is generally agreed that phloem capacity is not the limiting factor in photoassimilate movement between source and sink cells, cell uptake from the apoplast would be a possible rate-limiting point in the process of source to sink transfer. The kinetics of uptake have therefore been examined in source (leaf disc) and sink (root tip) explants of cotton. The results suggest some important differences in sucrose uptake kinetics which may be related to physiological function.

MATERIALS AND METHODS

Cotton seeds (*Gossypium hirsutum* cv Deltapine 70) were planted in 16.5-L plastic pails containing a mixture of equal parts peat moss, sand, and vermiculite and watered with a half-strength Hoagland solution. The resulting plants were raised to maturity in a refrigerated glass house.

Leaf Discs. Leaf discs, usually 0.28 cm^2, were punched from fully expanded cotton leaves, avoiding major veins. Leaf discs were washed briefly in dilute modified White's medium (19), then transferred to a full strength White's medium without label for 30 min to equilibrate the apoplast with media sugar. The uptake medium contained, in mmol/l: KNO_3, 3.0; $CaCl_2$, 1.03; $MgSO_4$, 3.16; KCl, 1.66; KH_2PO_4, 0.15; $MnSO_4$, 0.03; H_3BO_3, 0.024; $ZnSO_4$, 0.0094; KI, 0.0045; Fe ethylenediamine di-(*o*-hydroxyphenyl)acetate, 0.0027; Na_2MoO_4, 0.0010; and in mg/l: nicotinic acid, 0.5; thiamine·HCl, 0.1; pyridoxine·HCl, 0.1; and varying amounts of sucrose. Following this pretreatment, discs were transferred to labeled uptake solution and, following the uptake period, washed in identical solutions without label for 30 min, blotted, weighed, and extracted to determine radiolabel accumulation. All leaf disc experiments were conducted under laboratory lighting and temperature (about 23°C). Experiments with discs of various sizes, abraded leaf discs, and with leaf strips 2 mm wide showed no significant effect of leaf explant geometry upon sugar accumulation patterns, responses to inhibitors, pH, or monosaccharides, although rate of radiolabel accumulation was somewhat faster in abraded discs.

Root Tips. Seeds were surface sterilized with ethanol and NaOCl and germinated on moist filter paper in sterile Petri dishes for 2 d at 30°C in complete darkness. The 1-cm terminals of emerging roots were then aseptically excised and transferred to sterile culture media (identical to that used for leaf discs) in long-necked culture flasks which were shaken for 20 h in complete darkness at 30°C. Roots were next strained off and placed in 10 ml of aerated unlabeled culture media in 50-ml test tubes in a 30°C water bath for 30 min prior to radiolabel exposure. They were then transferred by straining to aerated labeled uptake solution for a radiolabel exposure which usually lasted 30 min and finally transferred to identical but nonlabeled media for a

30-min desorption period prior to determination of radiolabel incorporation.

Determination of Incorporated Radioactivity. Uniformly labeled [^{14}C]sucrose was obtained from New England Nuclear, Amersham, and Schwarz-Mann, and the radiochemical purity was checked by HPLC prior to use. The specific activity of uptake solutions ranged from 3.7×10^2 to 1.5×10^5 Bq/mol sucrose.

In some experiments, leaf discs were extracted and bleached in 2 ml 80% methanol by exposure to sunlight in a glasshouse for several days. Leaves were bleached in sealed glass scintillation vials until the methanol solution became clear. The methanol was then removed and combined with water-miscible scintillation cocktail (Beckman ReadySolv MP) before counting in a Beckman LS 7500 dpm scintillation counter[1] which automatically corrected for efficiency of counting and quenching. After milling to a fine powder by vortexing with glass beads in glass scintillation vials, the leaf disc residue was also placed in water-miscible scintillation fluid for determination of radioactivity in the alcohol-insoluble fraction of leaf tissue.

Following desorption and blotting, label in root tips was determined by placing them in 2 ml of 70% ethanol in sealed glass scintillation vials. The vials were then incubated in an oven for approximately 4 h at 70°C (11). Following heating, root tips were removed and placed in scintillation cocktail for determination of non-alcohol soluble radioactivity. Ethanol soluble radioactivity was determined following addition of scintillation cocktail to the ethanolic extract.

In other experiments, leaf or root tissue was combusted in a Harvey Instruments Biological Oxidizer (Hillsdale, NJ), and the evolved CO_2 was trapped in liquid scintillant. Sample radioactivity was then determined as for extracted samples. Extraction and combustion techniques gave identical determinations of radiolabel incorporation. Uptake, in all but those experiments with time as a variable, was for 30 min with the results expressed on a per hour basis.

To determine the short term fate of radiolabel from accumulated sucrose, plant material was ground in a Brinkmann Polytron in a 1:4 mixture of chloroform and ethanol to which water was added following homogenization to give two phases (10). The chloroform/water/ethanol-insoluble residue was extracted with hot (80°C) 70% ethanol to remove residual soluble carbohydrates. Following drying and powdering, starch in this residue was next solubilized with amyloglucosidase (13). Following several chloroform washes to remove residual lipids, phenolics were removed from the ethanol/water mixture by addition of a small amount of dilute HCOOH to protonate phenolics and filtering the fraction through reverse-phase minicolumns (Waters C-18 Sep-PAK). A neutral fraction, containing soluble carbohydrates, was prepared from the reverse-phase effluent by eluting it, sequentially, through Dowex 50W and Dowex 1 resins. After the neutral fraction was eluted from the Dowex resins with water, amino acids were eluted from the Dowex 50W column with 2 N NH_4OH and organic acids from the Dowex 1 column with 2 N HCOOH (2).

RESULTS

The rate of uptake of glucose, sucrose, and fructose by root cells from low (1 mM) sugar concentrations was found to exceed the rate of influx for these sugars into nonabraded leaf disc cells (data not shown). Fructose was found to accumulate faster than the other sugars in leaf cells from low sugar concentrations after uptake periods of approximately 1 h but at a slower rate into cultured root tips. Abraded leaf discs accumulated sugar at a faster rate, but the uptake pattern with increasing media concentration was identical to that with nonabraded leaf discs.

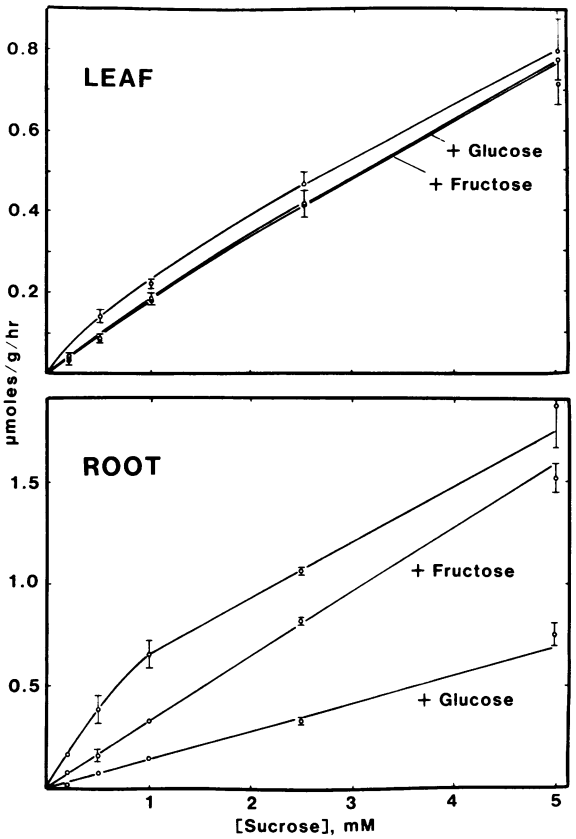

FIG. 1. Influence of 20 mM fructose or 20 mM glucose upon the influx of sucrose into root or leaf tissue from low apoplastic sucrose concentrations.

Both glucose and fructose decreased sucrose uptake by cotton roots (Fig. 1). Interestingly, the presence of these monosaccharides appears to eliminate the curved region of the sucrose uptake isotherm which is similar in shape to that referred to as the 'saturable' component in other systems (17, 18). As was found in *Beta* root (22), glucose causes a significantly greater depression of sucrose uptake than fructose. The inhibition of sucrose influx in cotton roots by glucose is more severe than that found in cotton hypocotyl (11). This depression of sucrose uptake by glucose was also found to not be transient and to be also caused by relatively low concentrations (2 mM) of glucose (not shown).

As has been found for other higher plant systems, the uptake of sucrose by both leaf and root tissue does not approach a maximal rate with increasing sugar concentration (Fig. 2). The complex nature of these kinetics are indicated by the nonlinearity of the Eadie-Hofstee plots in Figure 2. To determine whether these unusual kinetics are due, in part, to changing environmental osmotica in the various sucrose concentrations employed, sucrose uptake experiments were conducted in the presence of polyethylene glycol 6000 and mannitol (data not shown). The curves displayed in Figure 2 were not altered by either osmoticum at concentrations up to 200 mOsmol/l; however, solution osmolarities greater than 400 mOsmol/l did significantly change sucrose accumulation in both tissues.

Following a 30-min exposure to radiolabel, a large fraction of the incorporated sucrose from both tissues can be extracted with aqueous alcohol (Fig. 2), suggesting that it is not yet incorporated into polymers. Fractionation of the ethanol-soluble label accu-

[1] Mention of a trade name does not constitute a guarantee or warranty of the product by the United States Department of Agriculture and does not imply its approval to the exclusion of other products that may also be suitable.

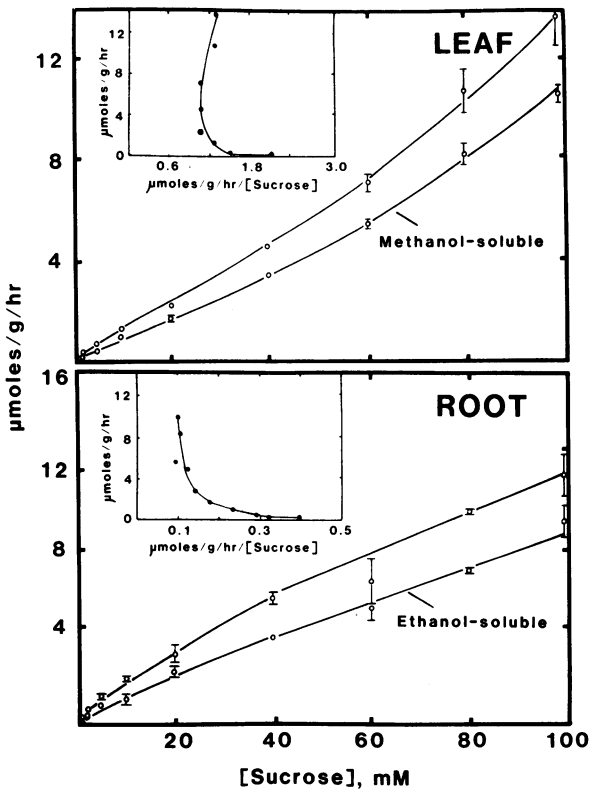

FIG. 2. Uptake of sucrose by cotton leaf disc and root tips from increasing apoplastic sucrose concentrations. Alcohol extractable values (see text) are also shown. Inset: Eadie-Hofstee transformation of uptake isotherms.

Table I. *Distribution of Label following [^{14}C]Sucrose Uptake by Cotton Leaf Disc and Cultured Root Tips*

The results represent the per cent label in each cell fraction resulting from exposure to ^{14}C-labeled sucrose (2 mM) for the times indicated, followed by a 30-min wash in identical but nonlabeled media prior to extraction.

Fraction	Uptake Time (min)		
	30	60	120
		%	
Root tips			
Lipid/phenolic	8.79	3.96	9.68
Amino acids	9.62	8.50	9.62
Organic acids	2.83	2.64	3.34
Amyloglucosidase-soluble	12.69	16.10	11.49
Neutral (sugars)	42.59	51.70	51.74
Insoluble	23.45	27.08	14.10
Leaf discs			
Lipid/phenolic	8.33	6.42	
Amino acids	4.97	4.46	
Organic acids	2.79	2.49	
Amyloglucosidase-soluble	3.16	3.68	
Neutral (sugars)	76.38	75.22	
Insoluble	4.35	7.70	

mulated by leaf cells showed that much of the short term label (about 75%) remained as neutral sugars (Table I). However, in root cell extracts, a significantly lower fraction (about 50%) was found in the same fraction. The difference in cell fraction labeling patterns between the two tissues appears to be due largely to a greater rate of incorporation of label from sucrose into polymers in root cells.

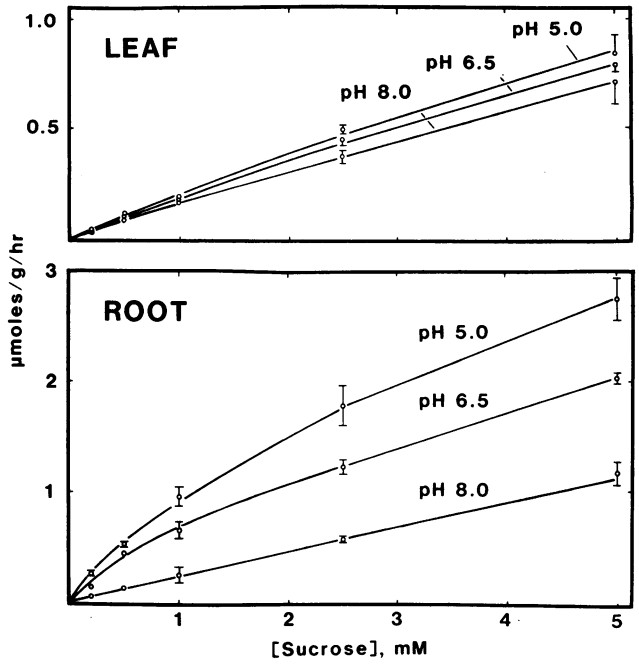

FIG. 3. Effect of pH upon sucrose uptake by leaf and root tissue from low concentrations of sucrose and 50 mM phosphate buffer.

Sucrose incorporation into both tissues from high solution concentrations (58 mM) was found to be independent of pH (not shown). In contrast, the maximal rate of influx from low sucrose concentrations (2 mM) occurs at media pH values less than 6.0. The effect of pH is more pronounced upon sucrose uptake by root than leaf cells (Fig. 3). As for *Beta* leaf discs (17), increasing pH eliminates the saturable component and decreases the slope of the linear sucrose uptake phase. Again, the patterns of sucrose uptake at differing pH values found for nonabraded and abraded leaf discs were identical (data not shown).

The uptake of sucrose by both systems can be inhibited by metabolic inhibitors (Figs. 4, 5). Uptake from low sucrose concentrations is more sensitive to inhibitors than that from higher sucrose concentrations. The uncoupling agent CCCP[2] is more effective than the nonpenetrating sulfhydryl reagents PHMB or PCMBS. Also, sucrose uptake by root cells is considerably more sensitive than uptake by both abraded and nonabraded leaf discs (not shown) to both types of inhibitors (Figs. 4, 5). Unlike other species (7), both PHMB and PCMBS appear to inhibit monosaccharide as well as sucrose accumulation into cotton root cells (Fig. 6); however, sucrose accumulation is more sensitive than monosaccharides to these inhibitors. The addition of PHMB, a mercurial very similar in structure and action to PCMBS (6, 14), during the pretreatment and uptake phases of the experiments, produces a similar pattern of inhibition of saccharide uptake but at an approximately 10-fold lower inhibitor concentration.

DISCUSSION

The uptake of sucrose from solutions by cotton leaf and root tissue appears to have some features in common with and some distinct differences from accumulation by other higher plant systems. Like other systems (3, 7, 11, 15, 17, 18, 20, 22), the uptake from low sucrose concentrations into both cotton tissues is pH dependent, with maximal uptake occurring near pH 5.

[2] Abbreviations: CCCP, carbonyl cyanide *m*-chlorophenylhydrazone; PHMB, *p*-hydroxymercuribenzoate; PCMBS, *p*-chloromercuriphenylsulfonic acid.

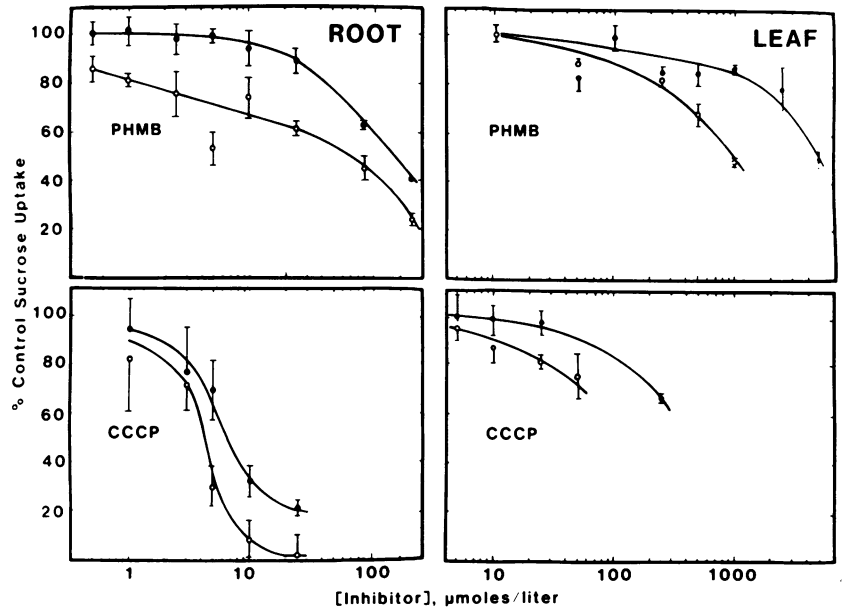

FIG. 4. Inhibition of root tip and leaf disc sucrose accumulation from 58 mM (●) and 2 mM (○) sucrose by the uncoupling agent CCCP and the organic mercurial PHMB. CCCP was present only during the uptake phases of experimentation; PHMB was present during both the pretreatment and uptake (both 30 min) phases.

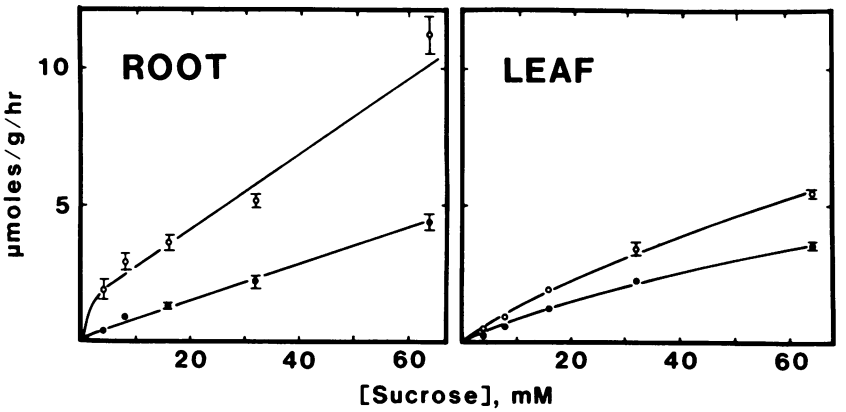

FIG. 5. Uptake of sucrose by cotton root tip and leaf disc following a 15-min pretreatment with 2 mM PCMBS and a 15-min wash in nonpoisoned media before uptake (●) or a 30-min pretreatment in nonpoisoned media before uptake (○).

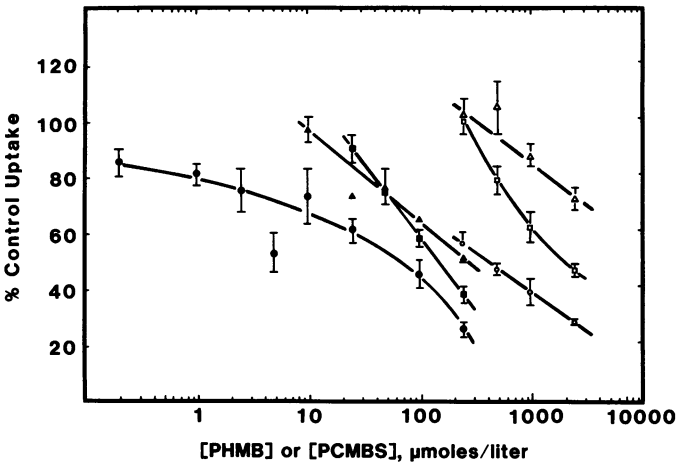

FIG. 6. Influence of various concentrations of the organic mercurials PCMBS (○, △, □) and PHMB (●, ▲, ■) on the influx of 2 mM sucrose (○, ●), glucose (△, ▲), or fructose (□, ■) into cotton root tips. PCMBS exposure as for Figure 5; PHMB exposure as for Figure 4.

Also, cotton tissue sucrose influx is inhibited by uncoupling agents and organic mercurials as has been found for a number of higher plant sugar accumulation systems (3, 7, 18). However, the cotton leaf and root systems have certain peculiarities. Pretreatment of cotton root tissue with PCMBS prior to radiolabeled sucrose exposure inhibits sucrose influx as in *Beta* leaf (6, 7), but unlike the *Beta* system mercurial exposure also sharply inhibits monosaccharide influx in cotton root. As has been shown in other systems (6), the patterns of sucrose uptake were found to be unchanged in abraded leaf discs compared to that where abrasion was eliminated. Uptake rates by abraded discs were somewhat faster, but the similarity of kinetics found in unabraded and abraded discs suggests that the diffusion limitation caused by the intact cuticle was not responsible for the inhibitor insensitivity, monosaccharide resistance, or lack of pH effect of sucrose uptake by leaf discs.

Previous work (17, 18, 21) demonstrated a biphasic dependence of sucrose accumulation upon sucrose concentration in tissues from other species. These kinetics are believed to consist of a saturable uptake component, which operates at low sucrose concentrations, and a superimposed nonsaturable, linear component. In other systems (7, 17, 18, 21), agents like CCCP (which uncouple ATP formation and inhibit ATPase energy transfer) and organic mercurials (which modify extracellular sulfhydryl groups necessary for influx [6, 7, 14]) were found to inhibit the saturable phase of sucrose uptake, exposing the linear component. The uptake of sucrose by cotton roots with increasing sucrose concentration is indeed more nearly linear following pretreatment with organic mercurials (Fig. 5), and the difference in uptake between this and inhibited uptake line and that with no inhibitor present would thus represent a saturable component, if such an interpretation holds for cotton root cells. However, in

the cotton root system, this saturable component is also eliminated by the addition of other sugars to the apoplastic solution (Fig. 1). Even an equal concentration of glucose, a physiologically probable condition, causes significant changes in cotton root sucrose influx kinetics. One possibility which should be considered to explain this behavior is that in cotton root extracellular invertase produces an influx of label in the form of sucrose and monosaccharides. The monosaccharide influx component would thus be diluted by any unlabeled monosaccharide in the uptake medium, providing data like that in Figure 1. Further, since free space invertase has been found to have an acidic pH optimum (4), such a hypothesis might explain both the pH optimum observed in sucrose uptake and the elimination of the saturable phase of sucrose accumulation found with increasing pH (Fig. 3).

As for other plant systems, organic mercurials inhibit sucrose accumulation by both cotton root and leaf disc cells (Fig. 4), but the interpretation of the leaf disc results as the elimination of a saturable component by the organic mercurial seems to be less appropriate than for the cotton root data (Fig. 5). The data for leaf disc sucrose uptake in the presence of PCMBS do not fall on a straight line, as is characteristic of corresponding data for root uptake, but a curved line which approximates the shape of the uninhibited curve. These leaf disc data show little evidence of a saturable component; *i.e.* the slopes of both the inhibited and noninhibited isotherm are observed to be nearly parallel as they approach zero sucrose concentration.

A portion of the difference between cotton root and leaf uptake kinetics may be related to their differing rates of conversion of sucrose into various cellular fractions (Table I). The relatively rapid movement of label from sucrose into other chemical forms in both tissues (*cf.* 21) suggests that the sugar uptake isotherms presented here are likely to involve not only movement of sugars across cell membranes but also the conversion of sucrose into other compounds. Cotton root cells exhibit a significantly faster rate of conversion of sucrose into polymers than leaf cells (Table I) and both tissues convert sucrose to other molecules more rapidly than many other plant systems (21). Such rapid polymerization of incoming sucrose may be necessary in cells which do not store large amounts of sucrose, such as those of cotton root, to produce a sink demand for incoming photosynthate.

Like many other plant tissues (1, 7), uptake by both tissues from low concentrations of sucrose appears to respond to the proton gradient between the cell and the apoplast. The sucrose accumulation responses of leaf and root cells to environmental pH are quite similar. Thus, if the two tissues utilize the proton gradient for sucrose influx, one might expect agents like CCCP which increase membrane permeability to H^+ (16), to have a similar effect upon tissue sucrose accumulation. In fact, however, sucrose uptake by cotton leaf cells is significantly more resistant to CCCP than that of root cells (Fig. 4).

Finally, in comparing data from these two tissues, it should be kept in mind that the cultured root tips were actively growing (*cf.* 19), but the leaf disc cells were taken from fully expanded leaves. Some of the differences noted might be traced to this difference. However, the fact that the uptake isotherms observed are not altered by a constant osmotic environment tends to minimize differences due strictly to cell expansion.

Acknowledgments—The criticism and advice of Drs. G. Guinn, R. Radin, R. Wyse, D. Doman, and W. S. Pierce are gratefully acknowledged.

LITERATURE CITED

1. Baker DA 1978 Proton co-transport of organic solutes by plant cells. New Phytol 81: 485–497
2. Canvin DT, H Beevers 1961 Sucrose synthesis from acetate in the germinating castor bean: Kinetics and pathway. J Biol Chem 236: 988–995
3. Delrot S 1981 Proton fluxes associated with sugar uptake in *Vicia faba* leaf tissues. Plant Physiol 68: 706–711
4. Eschrich W 1980 Free space invertase, its possible role in phloem unloading. Ber Dtsch Bot Ges 93: 363–378
5. Geiger DR 1975 Phloem loading. *In* MH Zimmermann, JA Milburn, eds, Encyclopedia of Plant Physiology, New Series, Vol 1. Springer-Verlag, Heidelberg, pp 395–431
6. Giaquinta RT 1976 Evidence for phloem loading from the apoplast. Plant Physiol 57: 872–875
7. Giaquinta RT 1979 Phloem loading of sucrose. Involvement of membrane ATPase and proton transport. Plant Physiol 63: 744–748
8. Giaquinta RT, W Lin, NL Sadler, VR Franceschi 1983 Pathway of phloem unloading of sucrose in corn roots. Plant Physiol 72: 362–367
9. Glasziou KT, KR Gayler 1972 Storage of sugars in stalks of sugarcane. Bot Rev 38: 471–490
10. Guinn G 1974 Abscission of cotton floral buds and bolls as influenced by factors affecting photosynthesis and respiration. Crop Sci 14: 291–293
11. Hampson SE, RS Loomis, DW Rains 1979 Characteristics of sugar uptake in hypocotyls of cotton. Plant Physiol 62: 846–850
12. Hampson SE, RS Loomis, DW Rains 1978 Regulation of sugar uptake in hypocotyls of cotton. Plant Physiol 62: 851–855
13. Hassig BE, RE Dickson 1979 Starch measurement in plant tissue using enzymic hydrolysis. Physiol Plant 47: 151–157
14. Hendrix DL, N Higinbotham 1974 Heavy metals and sulphhydryl reagents as probes of ion uptake in pea stem. *In* U Zimmermann, J Dainty, eds, Membrane Transport in Plants. Springer-Verlag, Heidelberg, pp 421–417
15. Komor E, M Rotter, W Tanner 1977 A proton-cotransport system in a higher plant: sucrose transport in *Ricinus communis*. Plant Sci Lett 9: 153–162
16. Le Blanc OH 1971 The effect of uncouplers of oxidative phosphorylation on lipid bilayer membranes: carbonyl cyanide *m*-chlorophenylhydrazone. J Membr Biol 4: 227–251
17. Maynard JW, WJ Lucas 1982a A reanalysis of the two-compartment phloem loading system in *Beta vulgaris*. Plant Physiol 69: 734–739
18. Maynard JW, WJ Lucas 1982b Sucrose and glucose uptake into *Beta vulgaris* leaf tissues. Plant Physiol 70: 1436–1443
19. Radin JW 1975 Differential regulation of nitrate reductase induction in roots and shoots of cotton. Plant Physiol 55: 178–182
20. Saftner RA, RE Wyse 1980 Alkali cation/sucrose co-transport in the root sink of sugar beet. Plant Physiol 66: 884–889
21. Thorne JR 1982 Characterization of the active sucrose transport system of immature soybean embryos. Plant Physiol 70: 953–958
22. Wyse R 1979 Sucrose uptake by sugar beet tap root tissue. Plant Physiol 64: 837–841