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Sucrose and Glucose Uptake into *Beta vulgaris* Leaf Tissues[1]

A CASE FOR GENERAL (APOPLASTIC) RETRIEVAL SYSTEMS

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ABSTRACT

Concentration curves for sugar and amino acid uptake by *Beta vulgaris* L. leaf tissues contained both a saturable and a linear component. Similarly shaped curves were obtained for influx of sucrose, glucose, and 3-*O*-methyl glucose by leaf discs, whole petiole slices, petiole segments containing pith tissue only, and petiole segments containing vascular bundles, although the tissues took up the various sugars via different proportions of saturable *versus* linear uptake. Two millimolar *p*-chloromercuribenzenesulfonic acid selectively inhibited the saturable component of sucrose uptake, but had almost no effect on the linear component. Uptake of glucose and 3-*O*-methyl glucose remained unaffected by *p*-chloromercuribenzenesulfonic acid treatment. Anoxia was found to inhibit the linear component of both sucrose and 3-*O*-methyl glucose influx, while the saturable component remained unaffected. The linear component of sucrose uptake was also competitively inhibited by maltose, as well as being selectively promoted by certain exposures to 5 millimolar *N*-ethylmaleimide, 2 micrograms per milliliter cycloheximide, and high levels of mannitol acting as osmoticum. These results support the proposal that the linear component is due to a process more complex than simple, or exchange, diffusion. It would also appear that the linear transport component utilizes a separate energy source than does the saturable component of sucrose influx.

Evidence for phloem loading from the apoplast was re-examined with respect to the present findings. Saturable sucrose uptake by minor vein tissues may represent retrieval of solute from the free space, which could explain the 'apoplastic loading' phenomenon.

Beta leaves and leaf discs readily absorb [^{14}C]sucrose from exogenous solutions (13, 28, 31). The assumption is frequently made that such uptake predominantly represents phloem loading, the process by which assimilate is accumulated in the sieve elements prior to translocation. However, it has been well-established that exogenous sucrose does not enter phloem tissues alone (1, 7, 27). Both Geiger (9) and Giaquinta (16) have pointed out that at high external concentrations, sucrose probably enters the cells of the mesophyll and vascular parenchyma, in addition to the se-cc[2] complex. Furthermore, numerous studies report that various other organic compounds are also readily taken up when exogenously supplied to leaves. These include other sugars, both hexoses and disaccharides (1, 7), and various amino acids (30).

Previous studies have shown that absorption of exogenous sucrose by source leaf discs closely resembles sucrose uptake by intact leaves (14, 28, 31). According to Sovonick *et al.* (31), sucrose uptake into the phloem occurs via two saturable (or biphasic) systems. However, the more recent work by Maynard and Lucas (28) indicates that this interpretation is inaccurate. Typical concentration isotherms for sucrose absorption into leaf tissue consist of both a saturable and a linear component, with total sucrose uptake being represented by the equation:

$$v = V_{max}S/(S + K_m) + kS, \quad (1)$$

where v is the rate, S is the sucrose concentration, V_{max} and K_m are the Michaelis-Menten constants, and k is the first-order rate coefficient (28). It is possible that the mechanisms (carriers?) responsible for the saturable and linear components of sucrose uptake are located on spatially distinct plasma membranes. For instance, the saturable process could represent the sucrose-proton cotransport that Giaquinta (14) envisions at the se-cc complex plasmalemma. The linear system may operate across the plasmalemma of the mesophyll tissue. Komor *et al.* (20) have proposed that a similar linear process in *Ricinus* seedlings may represent passive diffusion into mesophyll cells. Existing evidence does not allow an exact location of either component. Either process (or both) could conceivably operate from the apoplast at the se-cc membrane or at the phloem parenchyma cell membranes, or may represent uptake by mesophyll cells with eventual symplastic transport to the sieve elements.

Although there is a considerable body of evidence that is consistent with an apoplastic step in phloem loading (9, 16), the support is not unequivocal. The fate of exogenously supplied sucrose is, therefore, not completely clear. In the present study, we investigated whether foliar uptake of apoplastic sucrose represents a normal part of phloem loading, and not some other system. While the latter seems unlikely, it is possible that experimental conditions may exaggerate the effects of a process operating *in vivo* as, for example, a mechanism acting to retrieve solutes from the free space.

MATERIALS AND METHODS

Beta vulgaris L. (USH10 Lot 3102) plants were grown as described previously (28). Experiments were performed on tissues from mature leaves of 6-week-old plants.

Leaf discs 0.2 cm^2 in size and slices of petiole approximately 1 mm thick were excised under 20 mM citrate-phosphate buffer (pH 5) containing 20 mM $CaCl_2$. Further separation of petiole slices into 'pith' or 'vascular bundle' pieces (Fig. 2, inset) was done by punching out the 'semicircle' of pith tissue with a modified 3 mm diameter cork borer that had been bent to the shape of the vascular tissue. Resulting pith sections were examined to ensure that vascular bundles had not been included. The basic details of the 30-min pretreatment in unlabeled sugar and subsequent 30-min incubation in ^{14}C-sugars have been described previously (28). Briefly, all solutions contained 20 mM $CaCl_2$, Mes or citrate-

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[2] Abbreviations: se-cc, sieve element-companion cell; *k*, first-order rate coefficient; PCMBS, *p*-chloromercuribenzenesulfonic acid; NEM, *N*-ethylmaleimide; DTE, dithioerythritol; DNP, dinitrophenol.

phosphate buffer (pH 5), and sufficient mannitol to attain a uniform 500 milliosmoles of osmoticum. Arginine was obtained from New England Nuclear (Boston, MA); all other radioactive isotopes were obtained from ICN (Irvine, CA). Solution specific activities were 120 μCi/mmol for sucrose, glucose, leucine, and arginine, and 40 μCi/mmol for 3-*O*-methyl glucose. In experiments in which petiolar material was employed, transport rates were expressed on the basis of tissue fresh weight. Pith and vascular bundle sections were given the normal rinses in ice-cold nonradioactive experimental solution, and then the tissue, used in each substrate concentration, was subdivided into 30 mg lots. A Mettler PK300 electronic microbalance with a GA23 printer was used to obtain and record tissue weights. In some experiments, leaf discs were also weighed by this method.

Kinetic constants were calculated for both components of substrate influx. The first-order rate coefficient, k, was calculated as the slope of the linear regression equation for points on the total influx curve at or above 10 to 25 mM substrate, depending on where the curves approached linearity. Correlation coefficients (r^2) for the regression equations were generally very high, usually 0.998 or above, the lowest recorded being 0.979. The contribution of the linear process to total influx was estimated as k multiplied by the substrate concentration at each point, and this was subtracted from total influx at each point to give the rates due to the saturable component. The Michaelis-Menten constant, V_{max}, was then taken to represent the highest rate achieved by the saturable process. In order to avoid the problems associated with leaf-disc abrasion, we conducted experiments on unabraded discs. We wish to stress that because of this, the kinetic constants should be used only as indicators of relative transport components.

Sucrose and 3-*O*-methyl glucose uptake experiments were also performed in the absence of O_2. Anoxic conditions were established in a glove bag that was purged with a continual stream of nitrogen. Leaf discs and all experimental solutions were purged of O_2 and then held under anoxia for 45 min before the discs were transferred to [^{14}C]sucrose solutions that had also been purged of O_2. The subsequent 30-min incubation and two 5-min washes were also performed under anoxic conditions. Identical results were obtained under low-light conditions, (green safelight used as necessary to perform tissue manipulations) or in the dark.

In experiments in which we used various inhibitors, pretreatment in inhibitor solutions substituted for the pretreatment in unlabeled sugar solution. Except as noted, tissues were incubated in 2 mM PCMBS for 10 min (followed by two 4-min washes in citrate-phosphate buffer) or in 5 mM NEM for 5 min (followed by 5 min in 1 mM DTE to remove unreacted NEM). Further details are given in the appropriate figure legends.

Efflux analyses were performed as described previously (28). Two sets of 12 discs were incubated in 25 mM [^{14}C]sucrose for 2 h. One set was then transferred to 5 mM NEM solution (containing 25 mM [^{14}C]sucrose and 20 mM $CaCl_2$) for 5 min, and then to 1 mM DTE (also with sucrose and $CaCl_2$) for 5 min. Efflux into aliquots of Ca^{2+}-containing buffer was then followed for 3 h.

RESULTS

Kinetics of Sucrose Influx. In the extensive minor vein network of mature *Beta* leaves, relatively small sieve elements are associated with large companion and phloem parenchyma cells (9). In the petioles, the reverse is seen; large sieve elements are associated with smaller companion and phloem parenchyma cells. Large vascular bundles within the petiole are embedded in a nondifferentiated ground parenchyma which possesses little Chl (9). Thus, it was interesting to note that kinetic profiles for sucrose influx by leaf discs and by petiole slices exhibited the same general shape (Fig. 1). The k values for leaf discs and petiole slices were similar, being 0.064 and 0.057 μmol g^{-1} h^{-1} mM sucrose^{-1}, respectively, but the V_{max} for the saturable component in petioles was half that

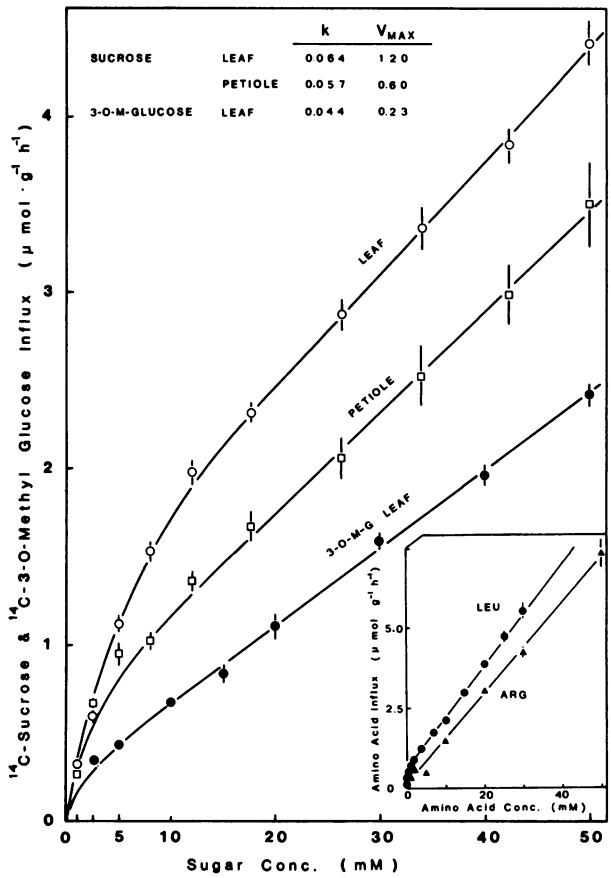

FIG. 1. Concentration dependence of sugar influx in *Beta* leaf discs and petiole slices. A 30-min preincubation in unlabeled sugar solutions was followed by 30-min in ^{14}C-labeled media. Each point represents the mean ± SE for two sets of seven replicates. Sucrose uptake by leaf discs (○); sucrose uptake by petiole slices (□); 3-*O*-methyl glucose uptake by leaf discs (●). Values for k are expressed as μmol g^{-1} h^{-1} mM sugar^{-1} and V_{max} as μmol g^{-1} h^{-1}. Inset, Amino acid influx into *Beta* leaf discs: leucine (●); arginine (▲).

of the leaf discs. This may reflect differences in the relative amounts of undamaged vascular tissue present or differences between the minor vein absorptive tissue and the vascular bundle conducting tissue.

The glucose derivative, 3-*O*-methyl glucose, cannot generally be metabolized by plant cells, although conversion to sugar phosphate may occur in certain species (3). Exogenous 3-*O*-methyl glucose is taken up only by mesophyll tissue in *Vicia* leaf discs (1) and is not loaded into *Beta* minor veins at a significant rate (7). Thus, while sucrose may be absorbed by both minor vein and mesophyll tissues (1, 6, 7), 3-*O*-methyl glucose is presently thought to accumulate in only one tissue. Nonetheless, uptake of 3-*O*-methyl glucose into leaf discs also exhibited kinetics similar to those of sucrose (Fig. 1). This is at variance with results from the *Ricinus* seedling system, in which 3-*O*-methyl glucose uptake was entirely linear (19). We also investigated the transport of leucine and arginine into *Beta* leaf discs and found similar kinetics (Fig. 1, inset).

In further experiments, petiole sections (cut transversely) were separated into pieces containing only pith parenchyma and pieces containing both pith and vascular bundles (Fig. 2, inset). Two-component concentration profiles were generated in both segments (Fig. 2). Sucrose influx gave rise to V_{max} values 3 times higher than those for uptake of 3-*O*-methyl glucose in both segments, while 3-*O*-methyl glucose influx gave rise to k values twice as high. Influx of 3-*O*-methyl glucose by bundle-containing pieces

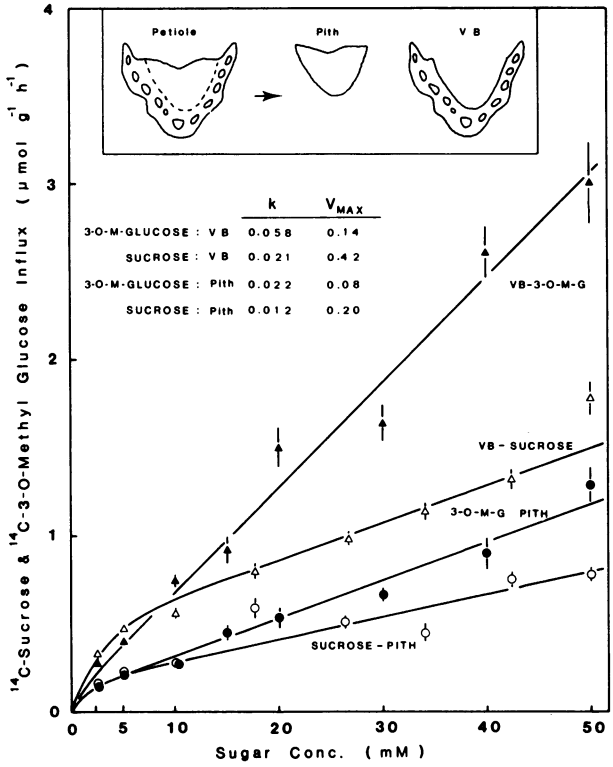

FIG. 2. Concentration dependence of sugar accumulation by dissected *Beta* petiole segments. Points represent the mean ± SE for seven replicates. Uptake of 3-*O*-methyl glucose by vascular bundle-containing (VB) segments (▲); sucrose uptake by VB segments (△); 3-*O*-methyl glucose uptake by pith parenchyma segments (●); sucrose uptake by pith segments (○) (k and V_{max} as in Fig. 1).

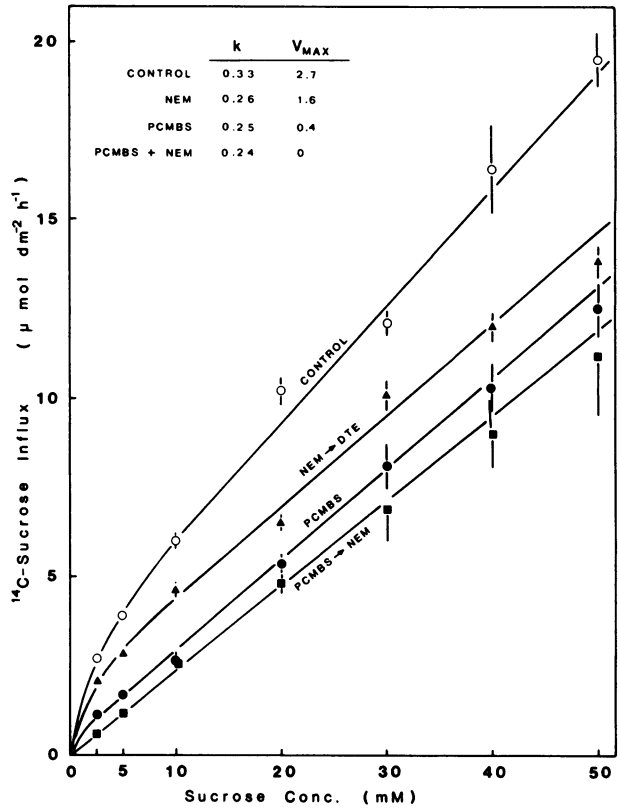

FIG. 3. Effect of sulfhydryl reagents on the concentration dependence of sucrose uptake. Control *Beta* leaf discs (○) were preincubated in citrate-phosphate buffer. PCMBS treatment (●) was for 10 min in 2 mM solutions. NEM treatment (▲) was for 5 min in 5 mM solutions followed by 5 min in 1 mM DTE. In other experiments, leaf discs were exposed to PCMBS and then NEM (■). All pretreatment solutions contained 5 mM sucrose, 1 mM $CaCl_2$, and 20 mM citrate-phosphate buffer (pH 5.0). Prior to introducing [^{14}C]sucrose, discs were given two 4-min washes in buffer solutions. Points represent the mean ± SE for three sets of six replicates. Values for k are expressed as μmol dm^{-2} h^{-1} mM sugar^{-1} and V_{max} as μmol dm^{-2} h^{-1}.

was essentially a linear function of concentration, while a saturable component made a large contribution to sucrose influx; the same features were observed for pith tissues. Thus, the saturable component cannot be entirely identified with the vascular bundles; however, since pith cells may be involved in sucrose storage, they could have the ability to accumulate sucrose selectively from the apoplast.

Effects of Sulfhydryl Reagents on the Kinetics of Sucrose Uptake. PCMBS is a nonpenetrating, reversible sulfhydryl reagent, while NEM penetrates and binds irreversibly to sulfhydryl groups. Both of these compounds have been shown to inhibit sucrose uptake into *Beta* leaves (13, 15). Five min in 5 mM NEM depressed both components of sucrose uptake below control values (Fig. 3). PCMBS-treated discs had similar k values to those of NEM-treated tissue, but an additional inhibition of the saturable component was evident. In two of four experiments of this nature, PCMBS-treated discs gave rise to linear kinetic profiles, while in the remainder a small component of saturable uptake (V_{max} = 0.2–0.4 μmol dm^{-2} h^{-1}) was still evident. Treatment with PCMBS followed by NEM abolished this remaining saturable component (Fig. 3).

Sequential treatment with sulfhydryl reagents demonstrated their additive action (Fig. 4). Preincubation with PCMBS followed by NEM gave rise to a linear profile, as noted above. However, exposure of similarly treated discs to DTE (which can remove bound PCMBS but not NEM) reversed the PCMBS inhibition, restoring, in part, the saturable component. Experiments of this nature demonstrated that NEM was able to bind to additional sites that PCMBS did not affect.

In subsequent experiments with NEM, we found that its effect on sucrose influx was complex (Fig. 5). Increasing the NEM (5 mM) period of exposure from 3 to 5 min increased the degree of inhibition, but tissues given a 10-min exposure began to show a 'recovery' in sucrose accumulation. Longer exposures, such as 30 min, appeared to increase the level of accumulated [^{14}C]sucrose. Only the linear transport component was involved in this increase; inhibition of the saturable component increased with exposure. (Fig. 5 contains the averaged results of four experiments conducted in December 1980. In later experiments using control, 5- and 30-min treatments, we did not find promotion of the 30-min treatment above that of the control; however, k values for the 30-min treated tissues were always greater than the 5-min treatment, and V_{max} values were lowered. It should also be noted that the high V_{max} values obtained from our earlier work, such as V_{max} of 16.7 μmol dm^{-2} h^{-1} reported for the control curve in Fig. 5, have since declined to the 2.2–5.0 μmol dm^{-2} h^{-1} level reported in our other data, reflecting differences in plant material or growth conditions that we cannot explain. However, V_{max} values obtained from field-grown material were within this latter range.)

NEM can also act as an inhibitor of protein synthesis (8). Cycloheximide, which is known to inhibit protein synthesis, was used in parallel experiments and was also found to selectively promote the linear component. The same effect was obtained with a 30-min treatment in cycloheximide followed by a 5-min wash as with a 5-min treatment followed by a 30-min wash, indicating that the time elapsed after exposure to cycloheximide may be the important factor, not length of treatment (Fig. 6).

It was possible, then, that long pretreatments in NEM promoted

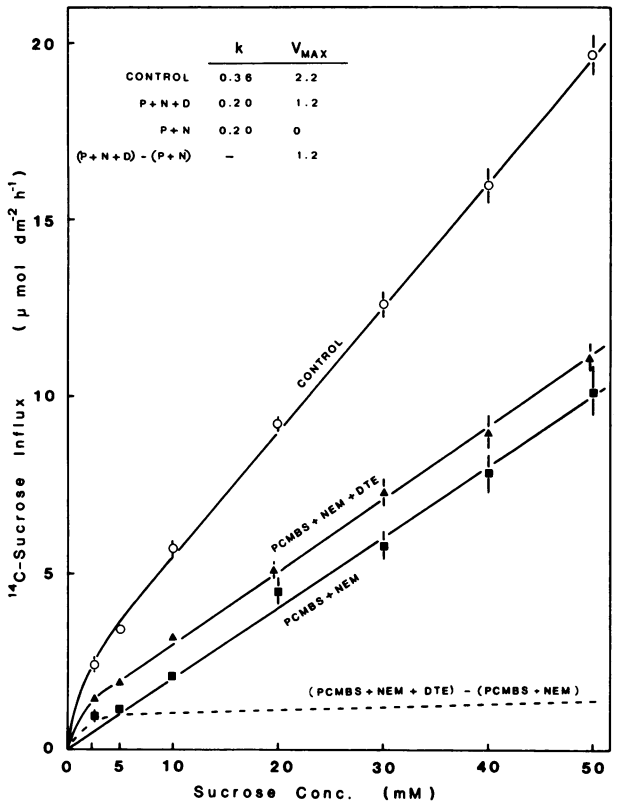

FIG. 4. Sequential addition of sulfhydryl reagents. Control *Beta* leaf discs were preincubated in 20 mM citrate-phosphate buffer (pH 5). PCMBS, NEM, and DTE treatments were as described in Figure 3. Points represent the mean ± SE for two sets of six replicates: control (○); PCMBS + NEM (■); PCMBS + NEM + DTE (▲) (k and V_{max} as in Fig. 3). The broken line was obtained by subtracting the PCMBS + NEM data from the values obtained in PCMBS + NEM + DTE experiments.

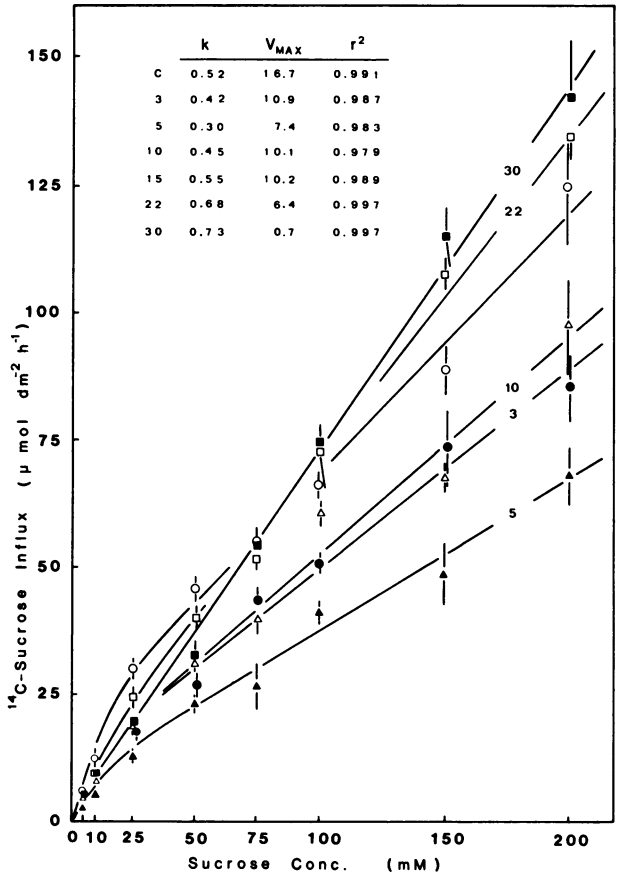

FIG. 5. Effect of increasing NEM exposure. *Beta* leaf discs were incubated in 5 mM NEM for varying times. A 5-min wash in 1 mM DTE was followed by a 30-min incubation in [^{14}C]sucrose. Points represent the mean ± SE for six replicate discs. Control (no exposure to NEM; ○); 3 min NEM (△); 5 min NEM (▲); 10 min NEM (●); 22 min NEM (□); 30 min NEM (■) (k and V_{max} as in Fig. 3).

linear uptake by allowing time for an inhibition of protein synthesis to take effect. Alternatively, long-term effects of NEM may have resulted from an increase in efflux of accumulated sugar (Table I). While both control- and NEM-treated sets of discs began the efflux period with similar dpm content, after 3 h of efflux there were significant differences in the radioactivity remaining. After the first 30 min, efflux became linear with time, with the NEM-treated discs losing about twice as much label as the control tissue.

Effects of Sulfhydryl Reagents on Hexose Uptake. Uptake of glucose and 3-*O*-methyl glucose by *Beta* leaf discs followed the characteristic saturable plus linear profile (Figs. 1 and 7). A comparison of the controls showed that both hexoses gave rise to similar k values, but uptake of glucose showed a larger saturable component than 3-*O*-methyl glucose. Uptake of neither hexose was particularly sensitive to PCMBS, which is in agreement with the earlier studies of Giaquinta (15). However, pretreatment with NEM significantly depressed the saturable component of 3-*O*-methyl glucose uptake (see also Ref. 15).

Further Manipulations of the Linear Component. The disaccharide maltose, which consists of two glucose units, appeared to compete with sucrose for uptake (Fig. 8). Inclusion of 100 mM maltose in experimental solutions (pretreatment and uptake) led to inhibition of both uptake components. An increase to 400 mM maltose gave rise to further inhibition of the linear component without an additional effect on the saturable system.

The inhibitor DNP, which uncouples phosphorylation in mitochondria and chloroplasts, has been used previously to demonstrate the energy-dependence of various sucrose uptake systems (14, 15, 30, 31). Pretreatment with 0.5 mM DNP led to inhibition of both components of sucrose accumulation (Table II), with the saturable system being the more sensitive.

Under anaerobic conditions, we found that the linear component of sucrose influx was reduced, while the saturable component appeared to be completely unaffected (Fig. 9). Since anoxia affects many aspects of cellular metabolism, we examined its influence on the influx of 3-*O*-methyl glucose; this molecule is generally believed to be a nonmetabolizable analog of glucose. As shown in Figure 10, anoxia also selectively inhibited the linear component of 3-*O*-methyl glucose accumulation.

According to earlier studies on sugar beet leaf tissues (11), cells of the mesophyll, phloem parenchyma, and se-cc complex contain equivalents of 500, 300, and 1000 milliosmoles mannitol, respectively. Hence, mesophyll and phloem parenchyma cells should be well-plasmolyzed when equilibrated over solutions of 1250 milliosmoles, although collapse of cell walls may prevent the complete disruption of the symplast (12). To determine whether sucrose and 3-*O*-methyl glucose transport exhibit differential osmotic sensitivity, uptake profiles for both sugars were obtained in the presence of 250 or 1250 milliosmoles. Pretreatment solutions did not contain sugar, and incubation in ^{14}C-sugar was shortened to 15 min in order to decrease possible effects of deplasmolysis (10). High levels of osmoticum promoted both components of sucrose uptake, with k and V_{max} being increased 148% and 145%, respectively. The k value for 3-*O*-methyl glucose uptake was also enhanced by 145%; however, the saturable transport component remained unaffected by high osmolality.

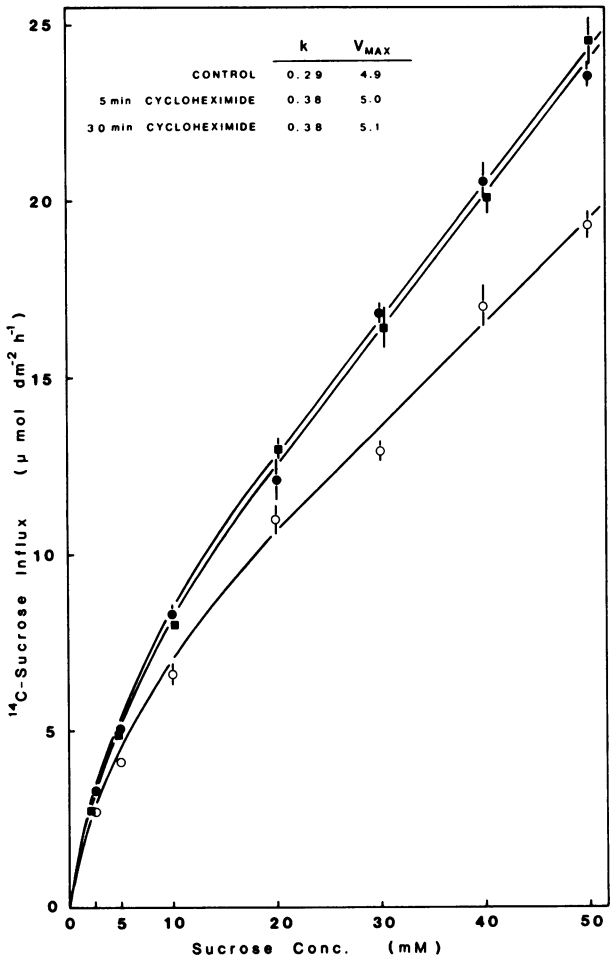

FIG. 6. Effect of cycloheximide on the concentration dependence of sucrose influx. Control sugar beet leaf discs (○) were preincubated in 20 mM citrate-phosphate buffer. Treatment in 2 μg/ml cycloheximide was for either 30 min, followed by a 5-min wash (■), or for 5 min, followed by a 30-min wash (●). Points represent the mean ± SE for seven replicates. (Units for k and V_{max} are as in Fig. 3).

Table I. *Effect of NEM on Efflux of Sucrose from Beta Leaf Discs*

Two sets of 12 discs were incubated in 25 mM [^{14}C]sucrose for 2 h. NEM-treated discs were then incubated in 5 mM NEM for 5 min, followed by 5 min in 1 mM DTE (solution details given in "Materials and Methods").

Efflux Time	Radioactivity Remaining in Discs	
	Control	NEM
h	*dpm (10^5)*	
0	1.83	1.79
0.25	1.19	1.08
0.5	1.09	1.00
1	1.01	0.88
2	0.89	0.68
3	0.82	0.51
	(0.095)[a]	(0.185)

[a] Loss of radioactivity after 1 h of efflux, in dpm h^{-1}.

DISCUSSION

Transport of Exogenous Substrates. Phloem tissue preferentially accumulates [^{14}C]sucrose and, consequently, it has been the tendency to equate exogenous sucrose uptake by leaf tissues with the actual process of phloem loading. Thus, the kinetics of [^{14}C] sucrose uptake have been interpreted with respect to the involvement of a H^+-sucrose cotransport system operating across the se-cc plasmalemma (see Refs. 9 and 16 for reviews of relevant literature). Fondy and Geiger (7) have shown, however, that in sugar beet tissue preincubated for 30 min in exogenously supplied 10 mM [^{14}C]sucrose, the majority of the label (60%) was found in the mesophyll tissue, with the remainder being either exported or present in the minor veins. Since our present experiments were conducted for 30 min, and we employed sucrose concentrations in the same range, it would appear that in the leaf the kinetic data represent transport of [^{14}C]sucrose across perhaps two different plasma membranes, those of the mesophyll and the se-cc complex.

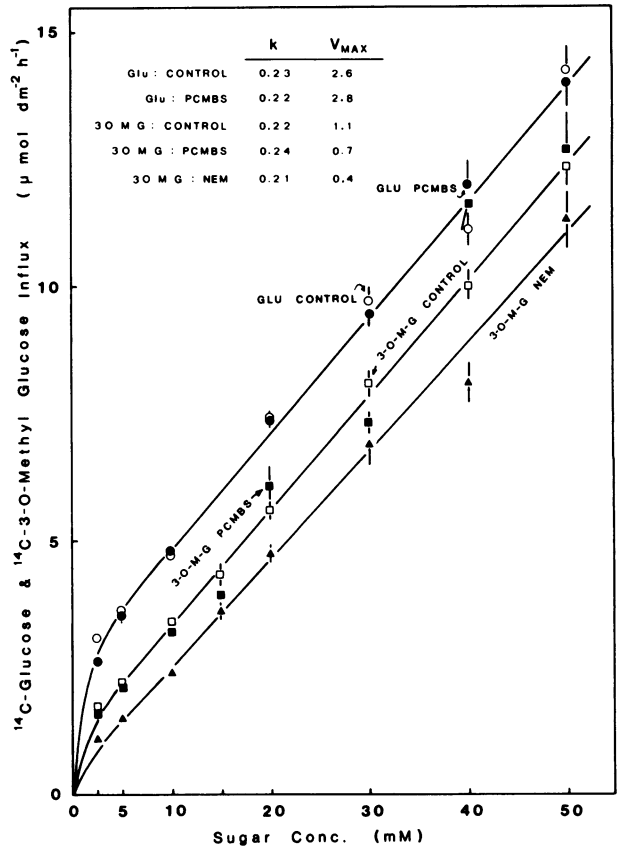

FIG. 7. Effect of sulfhydryl reagents on hexose influx in sugar beet leaf discs. Control, PCMBS, and NEM treatments are as described in Figure 3. Points for the glucose control (○), the glucose PCMBS (●), and the 3-*O*-methyl glucose control (□) represent mean ± SE for two sets of seven replicates; points for PCMBS-treated 3-*O*-methyl glucose (■) and the NEM-treated 3-*O*-methyl glucose (▲) represent the mean ± SE for seven replicates. (Units for k and V_{max} are as in Fig. 3.)

The kinetic curve for 3-*O*-methyl glucose transport into *Beta* leaf discs was found to be identical in form to that obtained for [^{14}C]sucrose (Fig. 1; see also Fig. 7 for glucose); *i.e.* uptake appears to result from the operation of a saturable and a linear transport component. The similarity between these kinetic curves gains in importance when one considers that uptake of 3-*O*-methyl glucose is confined to the metabolic space of the mesophyll cells (1, 7). This line of argumentation can be extended to our results obtained on sugar beet petioles. The petiolar vascular bundles are embedded in a parenchymatous tissue, not a chlorenchymatous mesophyll, yet sucrose uptake into vascular bundle segments still exhibited both saturable and linear components (Fig. 2). Of equal importance was the finding that sucrose uptake into petiole pith segments (free of phloem tissues) exhibited the same kinetics as found using the vascular tissue. The same trends were also found for 3-

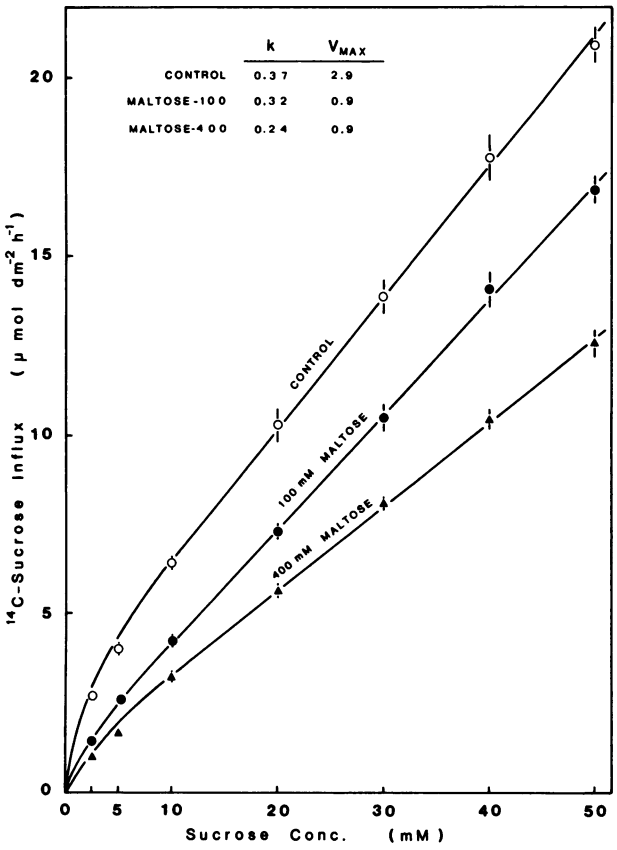

FIG. 8. Effect of maltose competition on the concentration dependence of sucrose influx. Sugar beet leaf discs were pretreated for 30 min in unlabeled sucrose and incubated for 30 min in ^{14}C-labeled sucrose containing either no (○), 100 mM (●), or 400 mM (▲) maltose. Solutions with 100 mM maltose contained 500 milliosmoles osmoticum, while those with 400 mM maltose contained 630 milliosmoles.

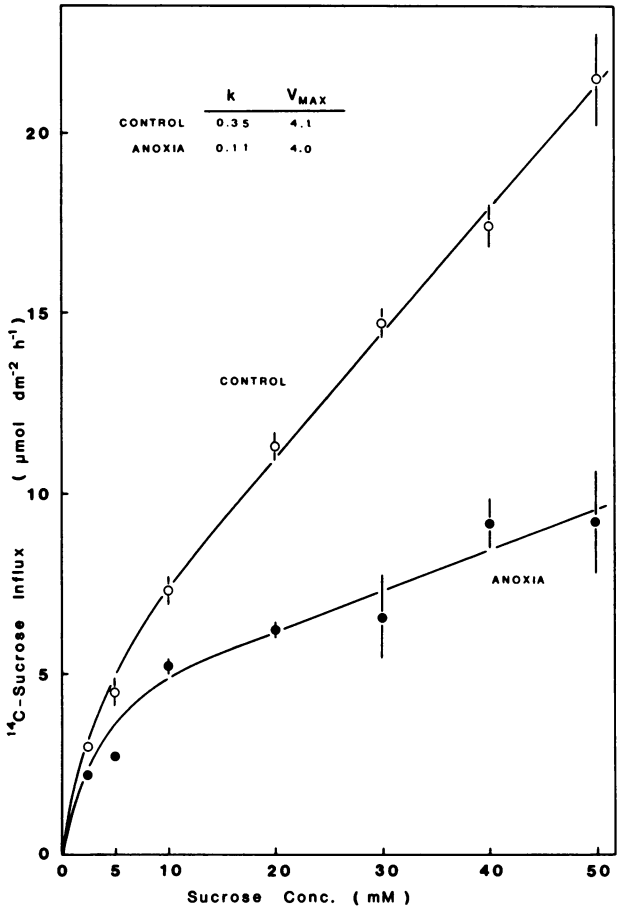

FIG. 9. Effect of anoxia on [^{14}C]sucrose influx. Tissue and experimental solutions were held under a nitrogen atmosphere for 45 min before the discs were transferred to [^{14}C]sucrose solutions. Incubation in labeled sugar for 30 min and two 5-min washes were also performed under anoxic conditions. Points represent the mean ± SE for six replicates.

Table II. *Effect of DNP on Sucrose Influx into Beta Leaf Discs*

The concentration dependence of uptake was determined for sucrose concentrations of 2.5, 5, 10, 20, 30, 40, and 50 mM, with two sets of seven replicate discs used at each concentration. Uptake curves were obtained for discs pretreated in 0.5 mM DNP for 0, 5, 10, and 60 min, and then incubated in [^{14}C]sucrose for 30 min. Kinetic parameters k and V_{max} were determined for the saturable and linear components of each curve.

DNP Treatment	k	V_{max}
min	*μmol dm^{-2} h^{-1} mM sucrose^{-1}*	*μmol dm^{-2} h^{-1}*
0	0.51 (100)[a]	4.8 (100)
5	0.42 (82)	2.4 (50)
10	0.39 (76)	1.8 (37)
60	0.37 (72)	1.5 (31)

[a] Numbers in parentheses, percent of control values.

O-methyl glucose uptake into petiole vascular bundle- and pith-segments.

It seems that the presence of a saturable and a linear component of transport is a ubiquitous feature of the plasma membranes of a rather wide variety of cell types within leaf tissues. This obviously complicates the interpretation of kinetic data with respect to the importance of a particular tissue (*e.g.* the se-cc complex) in terms of its contribution to the total uptake into the leaf or petiole. We contend that this makes it very difficult to equate uptake of exogenously supplied [^{14}C]sucrose to the process of phloem loading.

Nature of the Linear Component of Uptake. Reports of uptake systems consisting of a saturable and a linear component operating in parallel are becoming numerous in the literature. Examples of such systems involved in the transport of sugars include 3-*O*-methyl glucose uptake by *Riccia* thalli (5) and sucrose uptake in soybean cotyledons (23), *Vicia* leaves (2), *Ricinus* cotyledons (19, 20), *Beta* leaves (28), and dissected vascular bundles (33).

It is important to determine the nature and the location, within the leaf tissue, of this rather ubiquitous linear component. The sulfhydryl reagent, PCMBS, inhibited 85 to 100% of the saturable component of sucrose uptake, but had far less of an effect on the linear system (Fig. 3). Autoradiographs of *Vicia* leaf discs showed that PCMBS inhibited accumulation into the minor veins (1). These results are consistent with the effect of PCMBS reported by Giaquinta (13, 15) and, collectively, may indicate that the saturable component represents uptake via a sucrose-H^+ cotransport system. It appears that sucrose may be absorbed by minor veins, via a saturable process, and by parenchymatous tissues, via both saturable and linear processes. A possible nonsaturable contribution to minor vein uptake of sucrose may be masked by the larger linear influx into the surrounding parenchyma. In parenchymatous tissues, 3-*O*-methyl glucose is taken up primarily by a linear process, although a saturable component does make a contribution to the total uptake in *Beta*.

Based on the assumption that sucrose uptake occurs via sucrose-proton cotransport (14), Giaquinta has proposed that PCMBS effects in *Beta* are primarily due to inhibition of a plasmalemma ATPase (15). If linear sucrose uptake is carrier-mediated, as

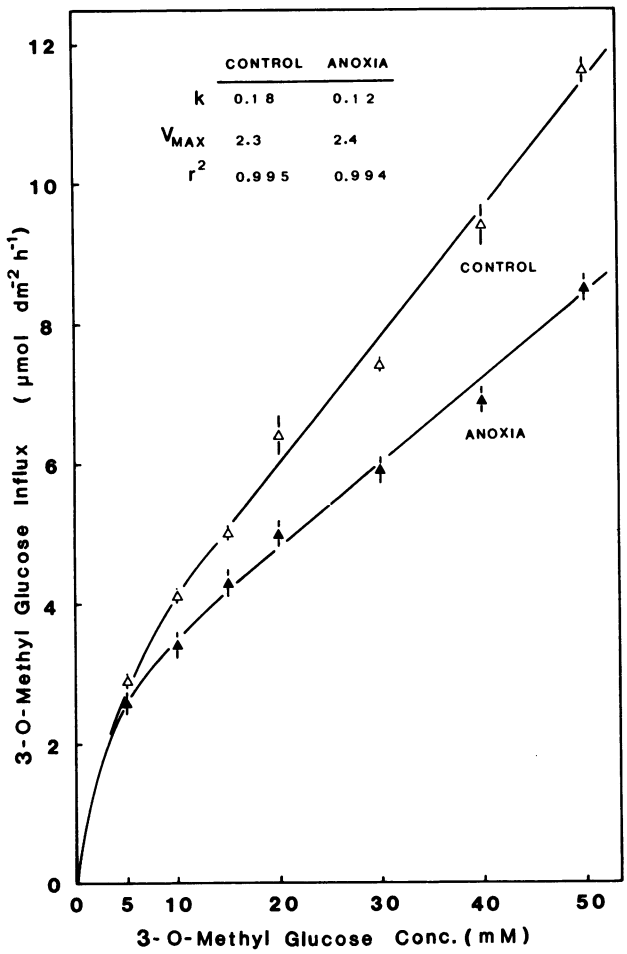

FIG. 10. Effect of anoxia on 3-*O*-methyl glucose influx. Tissue and experimental solutions were held under a nitrogen atmosphere for 45 min before the discs were transferred to [^{14}C]glucose solutions. Incubation in labeled sugar for 30 min and two 5-min washes were also performed under anoxic conditions. Points represent the mean ± SE for twelve replicates.

implied by maltose competition (Fig. 8), then interaction of PCMBS with the carrier system would affect both components. A relatively small degree of inhibition (20%) of *k* was seen (Fig. 3), but a much greater effect on the saturable component was observed (85–100%). Thus, if PCMBS acts on *Beta* plasmalemma ATPase activity, the linear component may utilize a different energy source compared to the saturable component (see also next section on anoxia).

The ability of NEM to enhance sucrose efflux (Table I) is not consistent with its promotion of linear uptake during long exposures, unless both phenomena represent some change in membrane permeability (22). Certain additional effects of NEM may be due to its ability to inhibit protein synthesis. In barley roots, cycloheximide treatment prevented protein synthesis but did not affect respiration, so that ATP levels actually increased, thereby causing a promotion of transport (26).

Effect of Anaerobiosis on [^{14}C]Sucrose Uptake. Selective inhibition of the linear component of sucrose uptake by anoxia is an extremely important finding (Fig. 9). Sovonick *et al.* (31) also reported an inhibitory effect of anoxia on sucrose uptake by *Beta* leaf discs. However, they analyzed their kinetic data in terms of the operation of two saturable transport components, presenting the effect of anoxia on the basis of changes in K_m and V_{max} of each 'saturable' system. This invalid kinetic approach led them to believe that anoxia was inhibiting both systems of transport, rather than the selective effect that we observed in the present study. We obtained the same selective inhibition of the linear uptake component of 3-*O*-methyl glucose under anaerobic conditions (Fig. 10). Since 3-*O*-methyl glucose is taken up only into mesophyll cells (7) and is not metabolized to any great extent, the similarity in effect between this molecule and that for sucrose suggests that the site of anoxia inhibition is at the plasmalemma and not at some location in the metabolic compartment(s). Thus, not only does the linear component appear to have a direct requirement for metabolic energy, but it appears to utilize a separate energy source than does the saturable component of sucrose influx. Possible energy sources include ATP from respiration, or perhaps reductant, such as NADH. Recently, a terminal oxidase which can accept electrons from exogenous NADH has been isolated from the plasmalemma of corn root protoplasts (24, 25). In this work, exogenous NADH was found to increase the O_2 consumption and the uptake of K^+ and phosphate in corn root segments. We cannot dismiss the possibility that a similar terminal oxidase may be involved in sucrose transport via the linear component.

The observation that the saturable component of sucrose (and glucose) influx was not affected by anoxia was quite unexpected. As discussed above, the saturable component of sucrose uptake is thought to represent sucrose-H^+ cotransport and may be operating across both phloem (se-cc complex and/or phloem parenchyma) and mesophyll plasma membranes. Due to the relatively high levels of ATP in the phloem sap (34), this tissue may not have experienced the same depletion of energy levels, under anoxia, as did the mesophyll tissue. If this were the case, the anoxia-insensitivity of the saturable component could be taken to indicate that this component resides almost entirely in the phloem. However, since the saturable component of 3-*O*-methyl glucose showed the same insensitivity to anoxia, and this sugar is only transported into mesophyll cells, this conclusion is suspect.

Anoxia, as a probe to study phloem physiology, could provide valuable insight into the operation of this complex system. Indeed there have been earlier reports that anoxic conditions inhibit phloem translocation (loading?). Leonard and Glen (21) found that a nitrogen atmosphere inhibited accumulation of label in the veins, as well as its transfer into the petioles of detached leaves of *Phaseolus vulgaris*. Similarly, Pickard *et al.* (29) reported that phloem translocation from intact moonflower leaves was stopped immediately after the imposition of anoxia. In a more extensive study on the effects of O_2 and phloem loading, Thorpe *et al.* (32) showed that loading in *Triticum aestivum* and *Panicum milioides* was decreased by approximately 70% within 1 to 6 min of anoxia application, but in *Zea mays* and *Panicum maximum* no reduction was observed. These authors ascribed these important differences to C_3 *versus* C_4 related properties. It will be of interest to ascertain whether species such as *Zea mays* have a linear component of sucrose uptake that is insensitive to anoxia.

Is the Apoplastic Step in Phloem Loading an Example of General Retrieval? Much debate has revolved around the *in vivo* movement of photosynthate from mesophyll chloroplasts into the se-cc complex. At present, an apoplastic step is favored, although the evidence in support of this is primarily from one plant: sugar beet (9, 16). If sugars normally enter the apoplast (7), then the plant must have some means of recovering this carbohydrate in order to prevent its unnecessary loss or build-up of conditions conducive to attack by pathogens. Fondy and Geiger (7) have suggested that "the mesophyll tissue may act as a collecting surface, reclaiming sucrose and hexoses which leak from the photosynthesizing cells." If this leakage does occur, and indeed there is experimental evidence showing that sugars (mainly sucrose, glucose, and fructose) do appear in the apoplast (7), then we can see no reason why sucrose would not leak from the se-cc complex. Since the sucrose concentration is high along the entire length of the translocation pathway, sucrose leakage may be a general phenomenon. Other proposals of retrieval mechanisms

have been proposed for phloem parenchyma cells (17) and certain thick-walled sieve elements in *Zea* minor veins (4). These modified cells possess an increased plasmalemma surface area, which may be set up to scavenge solutes from the apoplast and pass them on symplastically to the se-cc complex.

In experiments using exogenously applied sugar, we suggest that it is presently impossible to distinguish between 'apoplastic loading' and 'retrieval.' The distinction between the two becomes whether the mesophyll secretes sucrose only into the apoplast immediately surrounding the minor vein phloem, or whether sucrose leakage is an unavoidable phenomenon of the plasmalemma. It is possible that saturable uptake of sucrose by minor vein tissues represents the transport system that retrieves this sugar. While efflux of sugars from wheat and tobacco mesophyll protoplasts has been cited as direct support for an apoplastic step in translocation (18), this finding can also be interpreted as providing evidence for plasmalemma leakage, which may or may not be due to protoplast isolation.

Further evidence for apoplastic loading in *Beta* leaf tissues has been provided by use of the inhibitor PCMBS. Exogenous application of PCMBS inhibited uptake of [^{14}C]sucrose, as well as translocation of label supplied as both $^{14}CO_2$ and [^{14}C]sucrose, at inhibitor concentrations not affecting photosynthesis or respiration (13). If the saturable uptake seen in the present study does represent a retrieval or a loading mechanism, then its suppression would lead to the formation of a local sink at the site of inhibitor application: sieve elements would continue to leak, thus lowering their turgor, and a decrease in export would be seen from this site. Application of 10 mM PCMBS to intact *Beta* leaves led to an inhibition of [^{14}C]sucrose translocation from 11 to 6 μg carbon dm^{-2} min^{-1} after 60 min (13). Although Giaquinta (13) did not specify the sucrose concentration used, we found similar inhibition (*i.e.* approximately 50%) at lower sucrose concentrations (2.5–10 mM), and this can be explained as suppression of the saturable component (see Fig. 3).

CONCLUSIONS

Absorption of exogenous sucrose by leaf tissues represents more than loading into the phloem. The concentration (kinetic) curves contain both a saturable and a linear component. Identification of these transport components with the plasma membrane systems of the various leaf tissues still remains to be elucidated. In the present study, we provide further evidence that the linear component of uptake is not due to simple or exchange diffusion. This contention is supported by several lines of evidence: the linear component (a) operates against a concentration gradient, at least at lower concentrations; (b) is pH-dependent (28); (c) is dependent in part on metabolic energy (Table II; Fig. 9); (d) can be competitively inhibited (Fig. 8); (e) can be selectively promoted (Figs. 5 and 6); and (f) is enhanced by water stress-related changes in turgor (J. W. Maynard and W. J. Lucas, unpublished results). Finally, it has been shown that the exogenous sucrose acquired through the operation of this linear component is available for translocation (31).

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