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Transport of 3-*o*-Methylglucose Into and Out of Storage Cells of Daucus carota

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Abstract. The movement of labeled 3-*o*-methylglucose (MeG) into and out of thin carrot discs has been followed in order to gain information on sugar entry and exit mechanisms. Little or no metabolism of this derivative appears to occur in the tissue, since no products were detected either by chromatography or by analysis of respiratory CO_2.

The curve relating entry to external concentration deviated somewhat from a rectangular hyperbola but suggested a carrier mechanism. Glucose and MeG each competitively inhibited the uptake of the other. K_i for MeG was estimated to be 3 times the K_m for its uptake.

When discs incubated in MeG were transferred to H_2O, MeG lost to the solution from the Free Space was re-absorbed against a 7-fold concentration gradient.

The addition of unlabeled MeG or glucose to the medium surrounding discs which had been maintaining a ratio of internal to external MeG of 75:1 brought about release of stored isotope. This was probably not due to exchange diffusion *stricto sensu*.

Efflux of previously absorbed isotopic MeG into a medium containing unlabeled MeG or glucose was temperature-sensitive. The kinetics of efflux were complex and did not suggest a simple diffusion process related to overall MeG content. However there is evidence (including the falling rate of exit with time) that slow diffusion (or slow release from adsorption) contributed substantially to efflux. The source of this flow appeared to be neither the readily accessible Free Space nor the main storage compartment. Calculation indicated that the volume of this "slow diffusion compartment" might be about 1 % of the total volume of the discs.

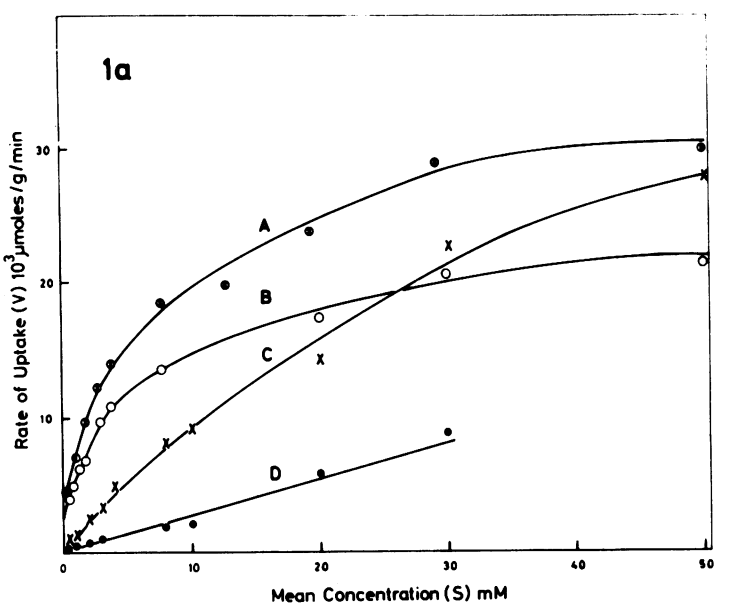
Transport mechanisms which convey sugars into a number of types of animal cell, as well as bacteria and yeast, have been the focus of much interest (see *e.g.* 19). Further, the opinion is gaining that the exit of sugars, notably from bacteria and yeast, is not due to simple diffusion but involves carriers and may be energy-requiring (see 7, 8, 9, 10). Though there are strong indications that entry mechanisms also exist in the cells of higher plants (1, 5, 6, 12, 14) investigators have been handicapped by 2 circumstances. Firstly, rate of uptake relative to rate of metabolism within the tissue is much slower than in many animal tissues. Secondly, there is a strong possibility that metabolism occurs to the outside of the diffusion barrier in which the transport mechanism is located. For instance, Reinhold and Filam (15) concluded that the sites where exogenous glucose is respired by sunflower hypocotyl segments lie within the "sugar Free Space" of the tissue. This would be the case if the site of the transport mechanism were, for example, the tonoplast. Where sugar uptake has been measured by its disappearance from the external solution, therefore, as in most of the work cited above, metabolism external to the transport mechanism may be gravely complicating the results.

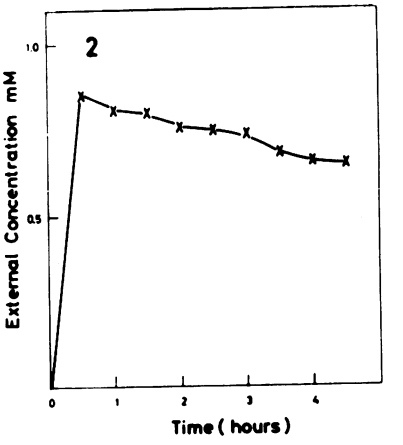
These difficulties can at least partly be overcome by recourse to sugar derivatives which are not metabolized by the cells. 3-*o*-Methylglucose has proved useful in work with animal cells (*e.g.* 13). Its use in the present study has enabled the nature of the exit process to be investigated as well as that of entry. This process has till now received little attention from plant physiologists.

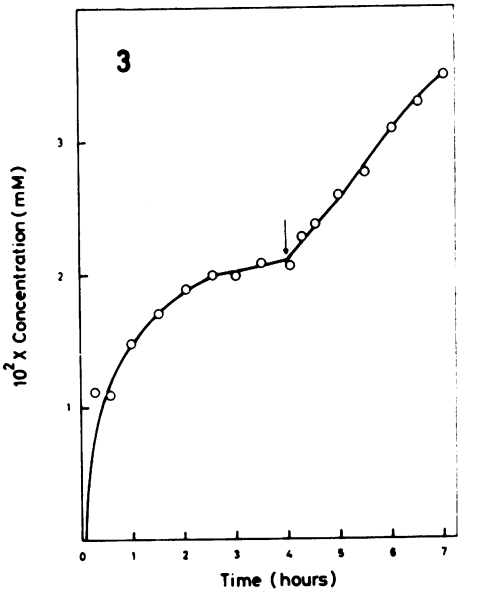
Materials and Methods

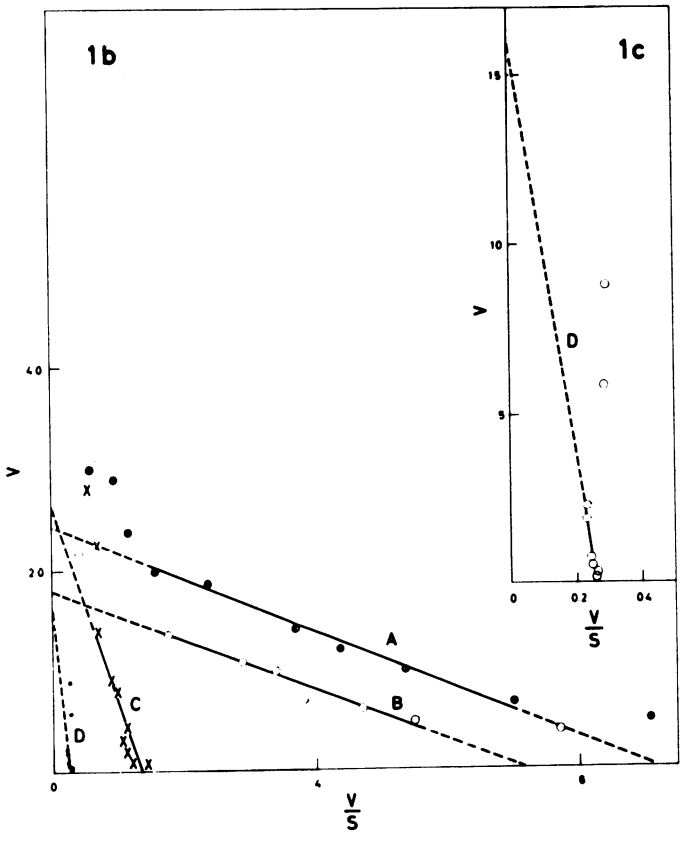
Plant Material. Discs 12 mm in diameter and 0.9 mm thick were cut from the xylem parenchyma of the storage roots of *Daucus carota* L. var. Nanti with the aid of a hand microtome. The discs were washed in running tap water before use in order to allow the leakiness characteristic of freshly-cut tissue to subside (16, 17). The washing period previously employed (14) was shortened to 24 to 30 hours to lessen the chance of bacterial contamination.

Influx Experiments. Weighed samples of discs were transferred to labeled 3-*o*-methylglucose solution contained as a shallow layer in tubes which were shaken continuously for the required incubation period. In most experiments (see individual experiments) the discs were washed in running tap-water for 30 minutes after incubation. Since it can be calculated (see 3, p 15) that a disc of this thickness will achieve 90 % diffusion equilibrium within 10 minutes (taking into account a tortuosity factor of 2, see 2) it is reasonable to assume that this treatment removed the sugar derivative present in the Free Space as usually envisaged (3, p 76–77). The discs were next extracted with 2 lots of 2.5 ml









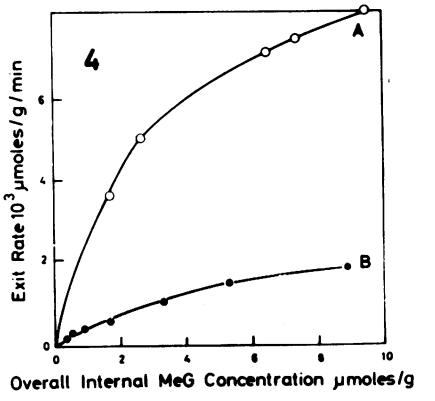


FIG. 1a. The relation between rate of uptake (V) and external concentration (S). Curve A) Glucose. Curve B) 3-*o*-Methylglucose (MeG). Curve C) Glucose in the presence of 40 mM MeG. Curve D) MeG in the presence of 40 mM glucose, 0.3 g samples of washed carrot discs placed in 2.5 ml medium for 3 hours.

FIG. 1b. The relation between V and V/S. Details as for figure 1a.

FIG. 1c. An enlargement of curve D in figure 1b.

hot ethanol (final conc, taking into account the tissue water, 80 %). Aliquots of the extract were counted in a Packard Tri-Carb liquid scintillation counter. In early experiments a dioxannaphthalene solvent system was used, containing 7 g PPO and 0.3 g dimethyl POPOP per liter. In later experiments the system was changed to toluene containing 33 % absolute ethanol, 4 g PPO and 0.1 g dimethyl POPOP per liter. Quenching was at the most 8 %.

Efflux Experiments. After incubation the discs were transferred, with or without washing (see text), either to distilled water, or to 20 mM unlabeled 3-*o*-methylglucose or glucose. After various intervals during which the flasks were continuously shaken, samples (0.2 ml) of the external solution were withdrawn to assess efflux, and were counted in the toluene scintillation mixture described above.

All experiments were carried out in duplicate or triplicate.

Chemicals. 3-*o*-methyl-^{14}C-D-glucose was obtained from Baird-Atomic Incorporated, and was checked chromatographically for purity. Carrier 3-*o*-methyl-D-glucopyranose, A grade, was supplied by Calbiochem.

Possible Metabolism of 3-O-Methylglucose Within the Tissue. To investigate whether the sugar derivative was metabolized, the alcoholic extracts were concentrated in a vacuum desiccator and chromatographed in the following 3 solvent systems: 1) Butanol-acetic acid-H_2O (5:1:4, upper layer), 2) Butanol-ethanol-H_2O (5:1:4, upper layer), 3) Butanol-pyridine-H_2O (6:4:3). The chromatograms were read on a Vanguard chromatogram scanner. No evidence for metabolism of 3-*o*-methylglucose was obtained. As an additional check, respiratory CO_2 evolved by carrot discs bathed in methyl-^{14}C-glucose solution was trapped in a mixture of 12 % ethanolamine and 88 % methanol, and counted by liquid scintillation. The count was not significantly different from the background.

Results

Relationship Between Rate of Uptake and Concentration in the External Medium. A typical example of a curve giving this relationship for 3-*o*-methylglucose (MeG) is shown in figure 1a (curve B). In order to examine how closely this curve approximates to a rectangular hyperbola, V (rate of uptake) has been plotted against the ratio $V:S$ (where S = external conc) in figure 1b. The relationship over this 100-fold concentration range is not strictly linear but is curved, departing from linearity most markedly at the higher concentrations. For comparison, curves obtained for isotopic glucose are also shown (curve A in figs 1a and b). The glucose curve has the same general form as that for MeG and in figure 1b is approximately parallel to the latter, indicating similar affinity for the transport system (see 4). It lies, however, above the MeG curve. Advantage may be taken of the fact that in both cases a good fit to a straight line is obtained over a large part of the range to estimate the approximate K_m (this fit is better than could be obtained by fitting 2 intersecting lines through the points, with the exception of those for the 2 or 3 highest conc. The latter possibly lie on a second straight line, again the lines are approximately parallel for the 2 substances). The calculated K_m for the concentration range where the linear relation holds is 2.4 mM for MeG and 2.6 mM for glucose.

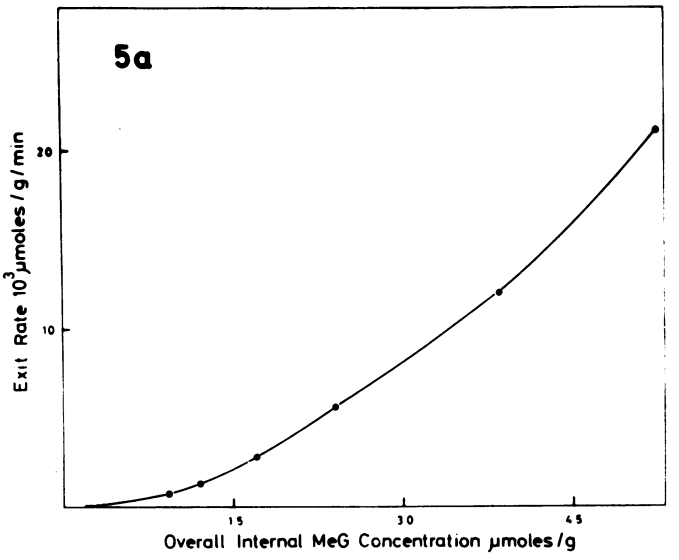
The mutually inhibiting effect exerted by each sugar on the uptake of the other is demonstrated by curves C and D in figures 1a and b. (An enlarged form of curve D is shown in fig 1c). Curves C and D give, respectively, the uptake of glucose in the presence of 40 mM MeG; and the uptake of MeG in the presence of 40 mM glucose. That the inhibition observed is competitive is indicated by 2 findings. Firstly, the degree of inhibition falls sharply as substrate concentration rises. (Curve C in fig 1a approaches and almost meets curve A. A similar fall in percentage inhibition with increasing conc is seen when D and B are compared.) Secondly, the marked increase in slope of curves C and D in figure 1b (see also fig 1c) as compared with the curves A and B respectively is diagnostic of competitive inhibition (see 4). The points for the 2 highest concentrations again depart from the straight lines in the case of both C and D, as they did in those of A and B.

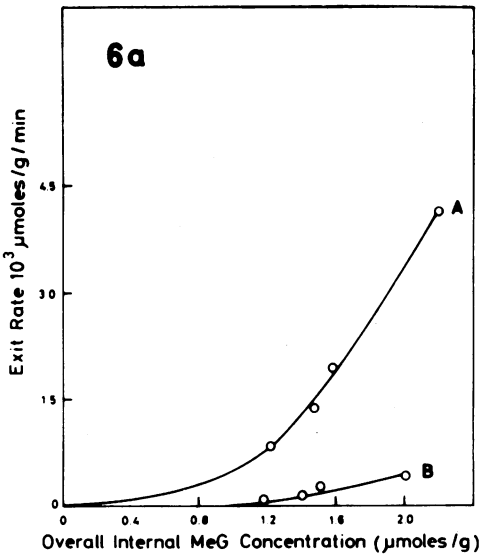
Uptake Against the Concentration Gradient. When carrot discs are allowed to take up MeG for a number of hours and are then very briefly rinsed

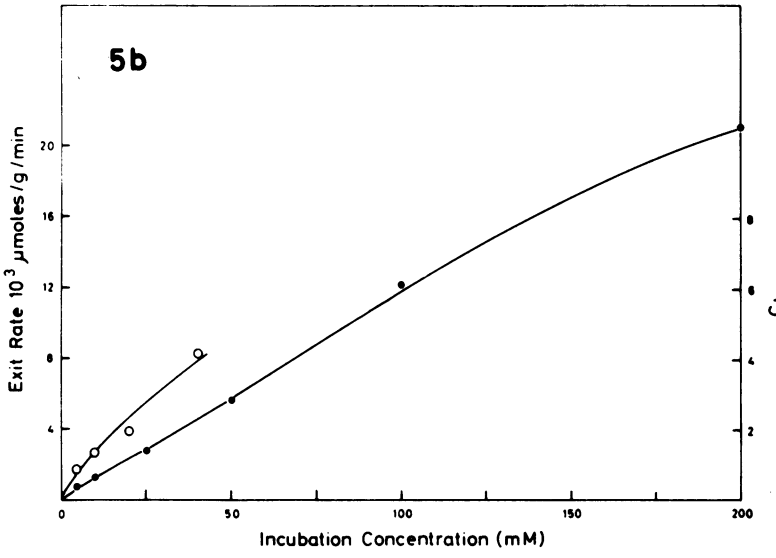
FIG. 2. The release and subsequent reabsorption of MeG by carrot discs after transfer from MeG to H_2O. 4.1 g samples of washed discs incubated in 20 mM MeG for 3 hours, then rinsed for 30 seconds and placed in 30 ml H_2O. MeG concentration in discs at the conclusion of the reabsorption period 4.5 μmole/g.

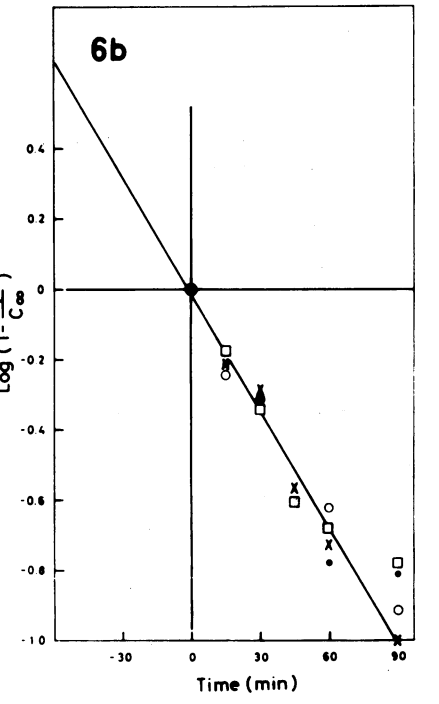
FIG. 3. Displacement of previously absorbed MeG by the addition of glucose to the medium. 3.39 g washed carrot discs incubated in 20 mM MeG for 3 hours, then washed for 30 minutes and transferred to 30 ml H_2O. At the arrow glucose added to medium to a final concentration of 20 mM.

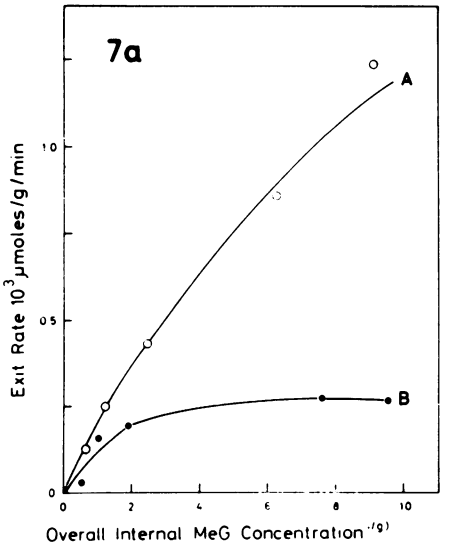
FIG. 4. The relation between rate of exit of MeG and internal MeG concentration. Curve A) Washed carrot discs incubated in 20 mM MeG for 2.5, 4.5, 17.0, 21.0, and 25.5 hours respectively, then washed for 30 minutes in H_2O and placed in 2.5 ml 20 mM MeG for 2 hours for efflux measurement. Curve B) Washed carrot discs incubated in 20 mM MeG for 0.17, 0.33, 0.66, 1.0, 2.5, 4.0, and 6.0 hours respectively, then washed in H_2O for 30 minutes and in 20 mM glucose for a further 90 minutes, after which placed in 2.5 ml 20 mM glucose for 2 hours for efflux measurement. Weight of carrot samples 0.35 g.

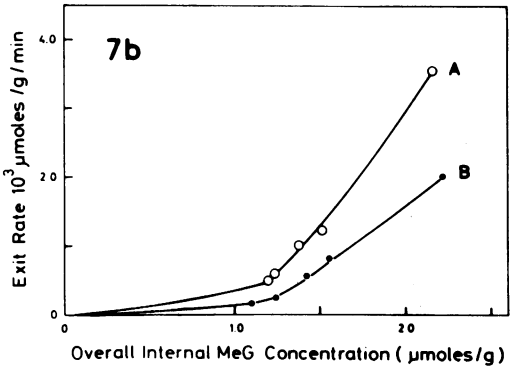

5a
Exit Rate 10^3 µmoles/g/min
Overall Internal MeG Concentration µmoles/g


6a
Exit Rate 10^3 µmoles/g/min
A
B
Overall Internal MeG Concentration (µmoles/g)


5b
Exit Rate 10^3 µmoles/g/min
Incubation Concentration (mM)


6b
Log (1- Ct/C∞)
Time (min)


7a
Exit Rate 10^3 µmoles/g/min
A
B
Overall Internal MeG Concentration (g)


7b
Exit Rate 10^3 µmoles/g/min
A
B
Overall Internal MeG Concentration (µmoles/g)

and transferred to water, MeG at first leaves the tissues (presumably from the Free Space) but is then steadily re-absorbed (fig 2). The average internal concentration in the tissue (taking 1 g tissue = 1 ml) was 7 times the external concentration at the close of the experiment illustrated.

Displacement of Absorbed 3-o-Methylglucose. If carrot discs, after incubation in MeG solution, are washed for 30 minutes in running water to remove the sugar derivative from the Free Space (see paragraph 2, Materials and Methods) and are then transferred to water, the amount of MeG appearing in the water is much smaller than in the previous experiment and reaches a steady level which might be interpreted as a "flux equilibrium". In the experiment shown in figure 3 the ratio of the average internal to the external concentration at this juncture was 75:1. If glucose, or unlabeled MeG, is now added to the external solution, the previously retained isotopic MeG is released into the solution at an approximately steady rate (fig 3).

Relationship Between Rate of Exit of 3-o-Methylglucose and Internal Concentration. A curve relating rate of exit of previously absorbed MeG (into an unlabeled MeG solution) to internal MeG concentration is shown in figure 4 (Curve A). This curve is characteristic for the situation where the range of internal concentrations has been obtained by incubating carrot slices for different lengths of time in a single concentration of labeled MeG. (Uptake was observed to be almost linear with time.) The curve does not indicate the linear relationship characteristic of a simple diffusion process.

When the range of internal concentrations is achieved, not as described in the last paragraph, but by incubating samples of tissue for a standard length of time in a range of concentrations of labeled MeG, a different type of curve is obtained. This effect has been checked in many experiments. An example of the curve is shown in figure 5a. A clue to the explanation of this remarkable concave curve is possibly to be found in figure 5b. Here efflux has been plotted, not against overall internal concentration as in figure 5a, but against the concentration of the solution in which the tissue had been incubated. The points from Curve A, figure 6a, similarly plotted, are also shown in figure 5b. An almost linear relationship is observed. This suggests that the MeG is contained in the tissue in more than 1 compartment, and that the concentration at the source of the efflux detected in figure 5a is almost directly proportional to the incubation concentration.

Figure 5 is based on total efflux measured over a 2-hour period. In other experiments the progress of efflux was followed and the latter was found to fall with time. Figure 6a summarizes such an experiment. Curve A gives efflux during the first 15 minutes after transfer of discs to unlabeled sugar solution (a 30 min washing period had as usual intervened between incubation and transfer) while curve B gives rate of exit from the same samples over the period 60 to 120 minutes after transfer. Data for the course of efflux over the experimental period may be found in figure 6b. They have been plotted in the manner shown for a reason which will be made plain in the Discussion. Since the drop in overall internal concentration over the period shown was only approximately 5 %, the sharply falling exit rate again suggests that the MeG is contained in more than 1 type of compartment in the tissue.

The results discussed in the last 2 paragraphs suggested the possibility that efflux during the first 1 to 2 hours might largely be due to diffusion from a relatively inaccessible region of the Free Space, or possibly to slow release from adsorption. It was therefore of importance to inspect the curve obtained under the conditions of figure 4 after this period

FIG. 5a. The relation between rate of exit of MeG and overall internal MeG concentration in the case where carrot samples had been incubated in a range of MeG concentrations as indicated in figure 5b. 0.35 washed carrot discs incubated in MeG for 3 hours, then washed for 30 minutes and placed in 2.5 ml 20 mM for 2 hours for efflux measurement.

FIG. 5b. The relation between rate of exit of MeG and incubation concentration. For details see figure 5a. The circles (right hand scale) are the points from Curve A, figure 6a, also plotted against incubation concentration.

FIG. 6a. The influence of time on the efflux curve. 4 g samples of washed carrot discs incubated for 3 hours in 25 ml 5, 10, 20, and 40 mM MeG respectively, then washed for 30 minutes and transferred to 30 ml 20 mM glucose. Aliquots withdrawn every 15 minutes to follow efflux. Curve A) Efflux during first 15 minutes after transfer. Curve B) Efflux 60 to 120 minutes after transfer.

FIG. 6b. The course of MeG efflux. $C_t:C_\infty$ is the ratio of the external MeG concentration at time t to that after 2 hours. For experimental details see figure 6a. Symbols □, ×, ○, and ● denote incubation concentrations 5, 10, 20, and 40 mM respectively.

FIG. 7. The effect of low temperature on the curve relating efflux to internal MeG concentration. Curve A) 20°. Curve B) 1 to 2°.

FIG. 7a. 0.3 g samples of washed carrot discs incubated in 30 mM MeG for 0.75, 1.5, 3.5, 10.5, and 13.5 hours respectively. Then washed in H_2O for 0.5 hour and in 30 mM glucose for further 1.5 hour, after which placed for 2 hours in 2.5 ml 30 mM glucose at 20° or at 1 to 2° for efflux measurements.

FIG. 7b. 0.3 g samples of washed carrot discs incubated for 3 hours in 6.25, 12.5, 25, 50, and 100 mM MeG respectively, then washed for 0.5 hour and placed for 2 hours in 20 mM glucose at 20° or at 1 to 2° for efflux measurements.

had elapsed. Curve B in figure 4 shows efflux out of discs which, after the usual 30 minute wash in tap water, had spent 90 minutes in unlabeled glucose solution, and had then been transferred to fresh glucose solution for efflux measurements. The curve still suggests saturation kinetics.

The Effect of Temperature on Efflux. The curve relating efflux to concentration was depressed by low temperature, both in the case where the tissue had been incubated for different lengths of time in 1 MeG concentration (fig 7a) and where it had been incubated in a range of concentrations (fig 7b). The effect is most marked on the right hand side of figure 7a, which, as explained in the Discussion, would be expected if, in addition to a temperature-sensitive component of efflux, there were a diffusion component issuing from a compartment other than the main storage compartment. Note also that the samples in figure 7a had been washed for an extra 1.5 hours.

Discussion

Although what appeared to be "uphill" uptake of glucose has been observed in higher plant cells before (6, 14) the evidence, as the former authors stressed, was not decisive. For if entering glucose passes in the first place into a compartment containing only a small proportion of the total glucose present in the cell, and if it is there metabolized, thus maintaining an inward diffusion gradient, then no "uphill" movement will in fact have taken place. Since in the present investigation no metabolism of MeG by the tissue could be detected, figure 2 provides unequivocal evidence for an active transport mechanism capable of conveying a glucose derivative against a 7-fold concentration gradient. Figure 3, moreover, shows that this mechanism could maintain a ratio of internal to external MeG of 75:1. The fact that unlabeled MeG, when added to the external solution, could "exchange" the stored ^{14}C, procuring its release into the medium, is further evidence in support of that given under Methods, that the sugar derivative existed inside the tissue unchanged, or at any rate in the form of a mobile molecule transported by the same system.

The kinetics of MeG uptake suggests a carrier mechanism, though it should be noted that the curve relating uptake to concentration deviates from a rectangular hyperbola. The simplest basic carrier scheme, *i.e.* reversible complex formation with a carrier that is in equilibrium with the substrate at both faces of the membrane (18, 19) thus cannot completely account for uptake by this tissue and requires modification. If there is an adsorption or slow diffusion component of uptake, as the efflux data seem to suggest, then this component would tend to produce deviation from a rectangular hyperbola in the direction observed in figures 1a and b. Only a small percentage of the total MeG taken up can be attributed to the diffusion component. however, since the overall internal concentration dropped by only 5 % during a 90-minute efflux period.

Figures 1a and b show that glucose inhibits the uptake of MeG more severely than does MeG the uptake of glucose, a fact that is of great interest in view of the parallel nature of the uptake curves A and B in figure 1b, suggesting very similar affinity for the carrier. (If several straight lines were fitted through the curves, then the line for MeG at the lower extreme of the conc scale would tend to be somewhat flatter than that for glucose, indicating a higher affinity in the case of the sugar derivative and thus emphasizing the anomaly of the inhibition picture.) Within the concentration range where the relationship between V and $V:S$ is linear, this effect can be quantitatively analyzed by making use of the Michaelis-Menten formulation

$$\frac{v_0}{v} = \frac{VS}{S + K_m} \cdot \frac{S + K_m + IK_m/K_i}{VS}$$

where v_0 and v are the rates of uptake in the absence and presence of inhibitor respectively, V is the maximum rate of uptake, S and I are substrate and inhibitor concentrations respectively, K_m the Michaelis constant for the substrate and K_i the inhibitor constant. It follows that

$$\frac{K_m}{K_i} = \left(\frac{v_0}{v} - 1\right) \cdot \frac{S + K_m}{I}$$

Considering the case where MeG is the substrate and glucose the inhibitor, the calculated values for this ratio at 3 substrate concentrations, 2, 8, and 20 mM, are 1.3, 1.2, and 1.1 respectively. But where glucose is the substrate and MeG the inhibitor the values for this ratio at the same 3 substrate concentrations are 0.41, 0.34, and 0.40 *i.e.* only about half the expected figure, the reciprocal of 1.2. If the K_m's for glucose and MeG are taken as 2.6 and 2.4 mM respectively, as suggested by figure 1b, then the average value for their K_i's are 2.0 and 6.8 respectively.

This apparently lesser affinity of MeG for the transport system when it is acting as inhibitor of glucose uptake as compared with that when it is itself being taken up, would be explained if glucose metabolism occurred to the outside of the membrane where the transport mechanism is located *e.g.* the tonoplast. Such metabolism would maintain the diffusion gradient for glucose into the cytoplasm, and the ^{14}C detected in the tissue at the end of an experiment would consist of the reaction products of the metabolic processes as well as the glucose transported into the vacuole. This would account for the glucose curve lying above that for MeG. For data on the products of glucose metabolism by carrot discs see Grant and Beevers (6). That at least part of the metabolism takes place to the outside of the principal diffusion barrier is indicated by the evidence provided earlier (15) that the sites where exogenously supplied glucose is respired lie in the "sugar Free Space" of the cell. If MeG competed with glucose for the transport system, but not (or

to a much lesser extent) for the enzymes bringing about metabolism, it would be expected that the apparent K_i for the sugar derivative would be larger than its K_m, as was observed. Discrepancies between K_m and K_i may also be deduced from figures reported for the uptake of various competing monosaccharides (6, 14). Here also the explanation may lie in differing affinities for metabolic enzymes external to the tonoplast.

An alternative explanation (6) should also be considered; that in addition to absorption sites at which mutual competition occurs between glucose and other sugars, there are sites which are specific for glucose transport.

Until very recently outward movement of sugar from cells capable of uptake against the concentration gradient was looked upon as due to a diffusional "leak" through an imperfect membrane. Today, however, there is evidence that this may not be the case for yeast and bacteria (7, 8, 9, 10) where carrier-mediated exit is now postulated. The present investigation has produced indications that exit of glucose from carrot slices may also not be due to simple diffusion. The first line of evidence is the displacement of previously absorbed MeG when glucose or MeG is added to the medium. The second is the non-linear kinetics of exit, especially as exemplified in the type of curve shown in figure 4. The third is the influence of temperature (fig 7). A Q_{10} of more than 2 has been regarded as evidence for carrier-mediated exit from yeast cells (see *e.g.* 9) though this criterion is not absolute (see 3, p 16). In the present investigation the Q_{10} is difficult to calculate because of uncertainty as to how much to allow for a diffusion component of efflux (see below) which may be masking the temperature-sensitive component. The relative contribution of the diffusion component should, however, be at a minimum on the right hand side of figure 7a, firstly because prolonged washing preceded the efflux measurements; and secondly because the samples on the right had been allowed to take up MeG for relatively long periods. The amount in the storage compartment (presumably the source of the temperature-sensitive efflux) will consequently have been large in comparison with that in the "diffusion compartment". The right-hand side of figure 7a suggests a Q_{10} of 2.

That slow diffusion from a relatively inaccessible region of the Free Space, or slow release from adsorption, may contribute substantially to the efflux measured in this study, is suggested by 2 findings: the sharply falling exit rate with time over a period when the overall internal concentration dropped very little, a result which indicates compartmentation of the tissue; and the conversion of the curve shown in figure 5a to a line with only slight convex curvature when incubation concentration is plotted along the horizontal axis. The latter result suggests that the concentration at the source of the efflux was directly proportioned to incubation concentration, unlike the overall concentration in the tissue as a whole. An estimate of the approximate size of this hypothetical "Slow Diffusion" compartment has been obtained as follows. The amount of MeG leaving this compartment during the 30-minute period of washing necessary for removal of sugar derivative from the surface of the discs and from the more readily accessible Free Space has been calculated by extrapolating the straight line shown in figure 6b back to $t = 30$. This value has been added to the amount leaving the tissue during the subsequent 120 minutes to give A, the total amount in the compartment (an overestimate which will give a maximum figure for the volume of the compartment, since not all the MeG leaving the tissue during the 120-min period will have come from this compartment). If the compartment was in diffusion equilibrium with the incubation medium at the end of the 2-hour incubation period, $A:S$ (where S = incubation conc) gives the volume of the compartment. The figures arrived at for the 4 incubation concentrations are 6, 4, 3, and 3 ml $\times 10^{-2}$, respectively. Since the total volume of the tissue was 4 ml (taking 1 g carrot = 1 ml) this indicates that the "Slow Diffusion" compartment accounted for about 1 % of the volume of the discs. One might visualize this compartment as the cytoplasm; or alternatively as certain types of cells in this heterogeneous tissue.

If the former, then the relative rates of diffusion into the cytoplasm and of carrier-mediated uptake into the vacuole must be such that the steady state concentration in the cytoplasm is near that of the external solution. Were this not the case, uptake would be related to external concentration by an oblique hyperbola, and the curves shown in figure 1b would deviate from straight lines in the direction opposite to that actually observed (see 15).

It is unlikely that the "exchange" of accumulated isotopic MeG by unlabeled MeG or glucose noted when the latter were added to the external medium (fig 3) was due to exchange diffusion in the strict sense (see 11). Since the latter is defined as movement of solute across a membrane in strict molecule-for-molecule exchange for a similar molecule moving in the opposite direction, and is thought to indicate a system where the carrier can only move in either direction when it is bound to the solute, net solute accumulation cannot occur. There is every indication that this is not true for carrot slices (6, 14). The "exchange" effect may have been due to competition for the pump in a "pump-and-leak" system, or alternatively to counter-transport. The latter phenomenon occurs where both entry and exit of sugar are carrier-mediated, and the efflux of accumulated sugar observed on addition of sugar to the external medium is due to the higher concentration of free carrier at the inner membrane face as compared with the outer (19). Whether counter-transport deserves serious consideration in the present instance will depend on whether a carrier-mediated step in fact proves to play an important role in exit.

Acknowledgment

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