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SUCROSE TRANSPORT AND HEXOSE RELEASE IN THE MAIZE SCUTELLUM*

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Key Word Index—*Zea mays*; Gramineae; maize; scutellum; sucrose transport; tonoplast; plasmalemma; hexose.

Abstract—Since hexoses readily diffuse from maize scutellum cells, it should be possible to detect them if they are produced during sucrose transport at the tonoplast or the plasmalemma. To test this idea, scutellum slices were placed in dinitrophenol (DNP) (which inhibits hexose utilization while greatly increasing utilization of vacuolar sucrose), and the utilization, uptake and leakage of sugars were measured. Only negligible amounts of hexose appeared in the DNP solution during a 5-hr incubation during which the slices metabolized 72 μmol of sucrose. Glucose and fructose, added at a concentration of 2 mM, were taken up by the slices at rates 33% and 14% (respectively) of the rate of vacuolar sucrose utilization. It is suggested, therefore, that sucrose transport at the tonoplast does not release free hexose into the cytoplasm. Sucrose transport at the plasmalemma was studied using DNP- and mannose-treated slices. During incubation of these slices in sucrose, the disappearance of sucrose resulted in the appearance of significant quantities of glucose and fructose in the bathing solution. Evidence is presented that sucrose is split into glucose and fructose during transport across the plasmalemma. It is concluded that free hexose is not normally a product of this splitting but is a result of an uncoupling in the transport system caused by the DNP or mannose treatments.

INTRODUCTION

A PREVIOUS paper[1] showed that maize scutellum slices bathed in 1 M sorbitol were unable to utilize or transport endogenous sucrose. However, 1 M sorbitol did not prevent transport and utilization of exogenous sucrose. When DNP was added to the sorbitol solution the slices rapidly metabolized vacuolar sucrose to the point of depletion (which evidently would require transport across the tonoplast) whereas the cytoplasmic sucrose remained untouched. From this study it was concluded that although the sucrose transport systems of tonoplast and plasmalemma are different, neither system releases free sucrose into the cytoplasm.

If free sucrose is not released, there are at least four other possibilities: sucrose phosphate, UDPglucose and fructose, glucose and fructose, and hexose phosphates. The release of sucrose phosphate would require direct phosphorylation of sucrose. This has not been demonstrated in higher plants, but a bacterial transport system appears to involve direct phosphorylation.[2] The release of UDP-glucose and fructose might result from sucrose synthetase (E.C. 2.4.1.13) acting as a sucrose carrier in the membranes. Similarly, glucose and fructose would be released by a carrier invertase (E.C. 3.2.1.26).[3] The release of hexose phosphates would require that sucrose splitting and hexose phosphorylation are coupled somehow in the transport system.

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[1] HUMPHREYS, T. E. (1973) *Phytochemistry* **12**, 1211.
[2] HENGSTENBERG, W., EGAN, J. B. and MORSE, M. L. (1967) *Proc. Nat. Acad. Sci. U.S.* **58**, 274.
[3] STORELLI, C., VOGEL, H. and SEMENZA, G. (1972) *FEBS Letters* **24**, 287.

This paper presents evidence that the sucrose transport system of the tonoplast does not release free hexose into the cytoplasm. Some free hexose is liberated during the uptake of exogenous sucrose, and it is suggested that this is a result of uncoupled transport at the plasmalemma.

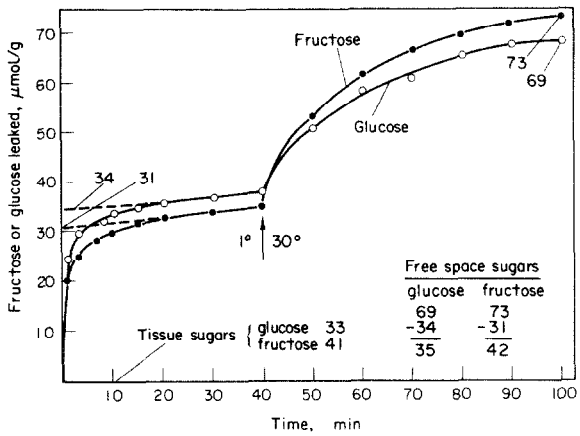

FIG. 1. EFFECT OF TEMPERATURE ON HEXOSE LEAKAGE.

Slices (1 g fr. wt) were placed in flasks containing 10 ml of DNP (5×10^{-4} M) plus 0·2 M glucose or fructose. The flasks were incubated at 30° for 35 min and then placed in an ice bath for 10 min. At the end of this period the bathing solution was removed, the slices were rinsed with 10 ml of DNP (5×10^{-4} M) at 1°, and 10 ml of DNP at 1° was added (time zero on the graph). After 40 min at 1°, the slices were rinsed with DNP at 1° and DNP at 1° added. The flasks were immediately placed in the 30° bath. Portions of the bathing solution were removed for hexose determinations at the times shown. Slices from one glucose and one fructose flask were killed after 10 min in DNP at 1° to determine tissue hexose. Slices from the glucose flask contained less than 1 μmol/g of fructose; slices from the fructose flask contained less than 3 μmol/g of glucose. Extracts of slices taken at the end of the 100 min leakage period contained less than 3 μmol of glucose. The term free space refers to intracellular free space.

RESULTS

Leakage of tissue hexose

Maize scutellum cells do not accumulate hexoses.[4] Hexoses readily penetrate the plasmalemma, but because the hexose space of the cell is small (12–25% of the tissue H_2O vol.) it appears that hexoses do not enter the vacuoles.[5] Hexose penetration of the plasmalemma is thought to occur by two processes: an active uptake which liberates hexose phosphates into the cytoplasm; and a passive diffusion through hydrophilic pores.[6] Therefore, when scutellum slices are placed in glucose or fructose solutions the hexoses enter the cytoplasm, and when these slices are transferred to water the hexoses rapidly leak out again. This is illustrated in Fig. 1. The slices were incubated at 30° in hexose plus DNP to load the cytoplasm with hexose; they were then transferred to a DNP solution at 1° (time zero in Fig. 1) and finally to DNP at 30°. DNP was used to increase the hexose space of the slices[5] and to inhibit hexose utilization.[6] The 1° incubation was used to differentiate between inter- and intracellular hexose space. Hexose whose leakage was strongly inhibited by low temperature is considered to have leaked from an intracellular space. The

[4] HUMPHREYS, T. E. and GARRARD, L. A. (1964) *Phytochemistry* **3**, 647.
[5] GARRARD, L. A. and HUMPHREYS, T. E. (1965) *Nature* **207**, 1095.
[6] WHITFIELD, J. H. and HUMPHREYS, T. E. (1972) *Phytochemistry* **11**, 2139.

same total amount of hexose as that shown in Fig. 1 leaked when the cold incubation was omitted.

Figure 1 shows that glucose or fructose leaked from the intracellular spaces in amounts about equal to the amounts of these sugars in the slices after removal of intercellular hexose. Extracts of slices taken at the end of the 100 min leakage period (Fig. 1) contained traces of glucose (<3 μmol/g). However, small quantities of glucose have always been found in extracts of thoroughly washed slices (e.g. see[4]) which perhaps indicates sucrose hydrolysis during extraction. It can be concluded from these data that essentially all tissue hexose is leakable. Therefore, if free hexose is produced during transport and metabolism of vacuolar or exogenous sucrose, some of it should leak into the bathing solution.

TABLE 1. GAS EXCHANGE, CALCULATED SUCROSE UTILIZATION AND MEASURED SUCROSE DISAPPEARANCE IN SUCTELLUM SLICES TREATED WITH DNP*

Process	Rate (μmol/g/hr)
CO_2 produced	88
O_2 consumed	36
Sucrose decrease in slices	14
Sucrose through glycolysis (calculated)†	16

* Slices were incubated in 0·1 M fructose for 3 hr at 30°, and then they were rinsed with water and placed in 10 ml of H_2O. After 10 min the bathing solution was replaced with fresh water; this step removed most of the free space fructose (see Fig. 1). After an additional 50 min in water the slices were used to measure sucrose disappearance and gas exchange. For sucrose disappearance, 1 g slices in 10 ml DNP (5×10^{-4} M) was used. For gas exchange, 0·2 g slices in 2 ml DNP (5×10^{-4} M) was used. Both processes were measured over a 5-hr period at 30°.

† This calculation was made on the assumptions that the entire gas exchange was a result of sucrose catabolism, that CO_2 produced in excess of O_2 consumed was a result of alcoholic fermentation,[7] and that the O_2 consumed resulted from the complete oxidation of sucrose.

Sucrose transport from the vacuoles

To test this idea on sucrose transport at the tonoplast, slices with high vacuolar (90–100 μmol/g) and low cytoplasmic (<10 μmol/g) sucrose levels were prepared (footnote, Table 1). These slices were placed in DNP, and the utilization, uptake and leakage of sugars were measured.

That sucrose utilization involved sucrose splitting (and, therefore, the possible production of free hexose) was indicated by the gas exchange of the slices which was consistent with alcoholic fermentation as the main metabolic pathway for sucrose utilization (Table 1 and [7]). The rate of sucrose utilization calculated from the gas exchange data was close to the measured rate of sucrose disappearance. This calculation indicates that there was no net loss of hexose to the bathing solution; and, therefore, if free hexoses were produced during transport and breakdown of sucrose they must have been phosphorylated as rapidly as they were formed.

[7] GARRARD, L. A. and HUMPHREYS, T. E. (1968) *Phytochemistry* 7, 1949.

In the experiment of Fig. 2 slices were incubated in DNP or in DNP plus 2 mM glucose or 2 mM fructose. The slices catabolized an average of 72 μmol of sucrose during the 5-hr incubation (see Table 1) irrespective of the presence of 2 mM hexose. From the results of Fig. 2 it appears unlikely that UDP glucose and fructose or glucose and fructose could be intermediates in the transport and catabolism of vacuolar sucrose without considerable loss of hexose to the bathing solution. This conclusion is based on the following observations: (1) only small amounts of hexose appeared in the bathing solution when the slices were placed in DNP alone (Fig. 2), and this probably arose from the splitting of sucrose that leaked from the slices in small amounts (see Fig. 3); (2) judging from the slow rate of fructose uptake, if 72 μmol of fructose were produced during sucrose transport at the tonoplast, quantities of it should leak into the bathing solution (Figs. 1 and 2, Table 1); (3) the slow rate of fructose utilization (Fig. 2) was not due to its inability to penetrate the plasmalemma since Fig. 1 shows that intracellular glucose and fructose leaked from the cells at about the same rate and that leakage continued until the tissue was nearly devoid of hexose; (4) in a separate experiment slices were incubated in DNP plus both glucose and fructose. Fructose uptake was inhibited about 50% by the presence of glucose whereas glucose uptake was not affected by fructose. In the presence of exogenous glucose, the utilization of any fructose produced at the tonoplast should also be inhibited; this would increase its chances of leaking to the cell exterior. However, extra fructose leakage was not observed when glucose was added to the bathing solution (Fig. 2).

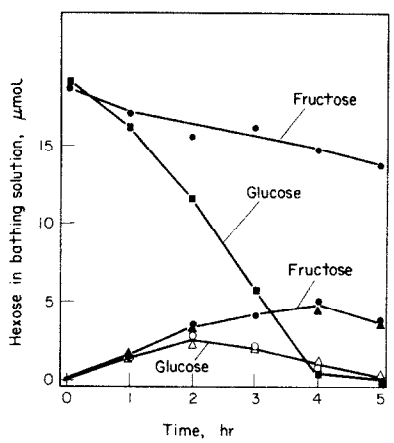

FIG. 2. HEXOSE LEAKAGE AND UPTAKE DURING INCUBATION OF SLICES IN DNP.
Slices (1 g fr. wt) prepared as in Table 1 were placed in flasks containing DNP (5×10^{-4} M) or DNP plus 2 mM glucose or fructose. Top curve: fructose uptake. Middle curve: glucose uptake. Bottom two curves: fructose leakage into DNP or DNP plus glucose and glucose leakage into DNP or DNP plus fructose.

Sucrose transport at the plasmalemma

In contrast to tonoplast transport, it was possible to demonstrate hexose release in significant amounts during sucrose transport at the plasmalemma. When slices were incubated in DNP plus 2 mM sucrose, the disappearance of sucrose from the bathing solution was accompanied by the appearance of fructose and glucose (Fig. 3). The glucose concentration of the bathing solution reached a maximum at 2 hr, and glucose was then absorbed; fructose continued to be released throughout the 5-hr incubation. Therefore, the

bathing solution, which initially contained 20 μmol sucrose, contained 15 μmol of fructose and only traces of glucose and sucrose after 5 hr. The glucose and fructose released into the bathing solution during the first 2 hr completely accounted for the sucrose that disappeared, and during the entire 5-hr incubation the fructose released accounted for about 75% of the sucrose that disappeared.

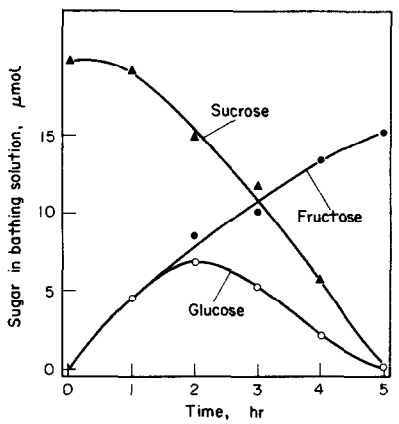

FIG. 3. SUCROSE DISAPPEARANCE AND HEXOSE APPEARANCE DURING INCUBATION OF SLICES IN DNP PLUS SUCROSE.
Slices (1 g fr. wt) prepared as in Table 1 were placed in flasks containing DNP (5×10^{-4} M) plus 2 mM sucrose. Portions of the bathing solutions were removed for sugar analyses at the times shown.

In the experiment of Table 2, slices were incubated in DNP plus 2, 4 or 6 mM sucrose. At each concentration of sucrose the glucose content of the bathing solution reached a maximum at 2 hr as in Fig. 3, and during the following 3-hr period glucose disappeared at the same rate in all flasks irrespective of sucrose concentration. In contrast, the rate of sucrose disappearance from 6 mM sucrose was more than twice the rate from 2 mM sucrose. Evidently, the process by which sucrose disappeared during the last 3 hr of the incubation released little if any freely diffusible glucose. However, the process did release fructose in quantities that were roughly 50% of the amount of sucrose that disappeared (Table 2). This suggests that splitting of sucrose is part of the uptake process. The release of glucose and fructose early in the sucrose incubation and the release of only fructose later on (Fig. 3) suggest that during normal transport there is tight coupling between the splitting of sucrose and the utilization (phosphorylation?) of the resulting hexoses. This coupling, initially disrupted by DNP, is completely restored after a few hr for glucose but only partially restored for fructose. Note that during the last 3 hr of the incubation (Fig. 3, Table 2) sucrose disappeared at about twice the rate of fructose appearance.

It should be noted here that the previously reported[6] inhibition (>90%) of sucrose uptake by DNP was obtained during the first 90 min of uptake and that uptake, not "disappearance", was measured (i.e. sucrose disappearance accompanied by the appearance of glucose is not counted as uptake, see Fig. 3).

Additional evidence in support of the idea that splitting of sucrose is part of the uptake process was obtained with mannose-treated slices. Mannose treatment consisted of incubating the slices in mannose for 1 hr followed by a short water wash (see Table 3). In the

experiments reported here, the utilization of endogenous sucrose was completely stopped during the 5-hr experimental period following mannose treatment: the small decrease in tissue sucrose (3–4 μmol) after 5 hr in water resulted from sucrose leakage (see footnote, Table 3). Mannose treatment also causes an intracellular accumulation of mannose-6-phosphate (M-6-P)[4] and a marked drop in ATP content of the slices.[8] Nevertheless, sugar uptake into mannose-treated slices, although inhibited, was surprisingly rapid. Glucose (2 mM) was taken up at a rate of about 4 μmol/g hr. For the first 2 hr fructose was also taken up at this rate, but the rate then fell to 2 μmol/g hr. More importantly, when glucose and fructose were added together the same rates of uptake were obtained as when they were added to separate flasks.

TABLE 2. CHANGES IN THE SUGAR CONTENT OF THE BATHING SOLUTIONS AT THREE SUCROSE CONCENTRATIONS*

Initial sucrose concn (mM)	Glucose concn at 2 hr peak (mM)	Change in sugar content, 2–5 hr (μmol)		
		Glucose	Sucrose	Fructose
2	0·78	−7·4	−11·7	+4·4
4	0·94	−7·6	−18·7	+10·4
6	1·10	−7·5	−27·6	+13·4

* Scutellum slices (1 g fr. wt) prepared as in Table 1 were placed in flasks containing 10 ml of sucrose plus 5×10^{-4} M DNP. Portions of the bathing solution were removed for sugar analysis each hr for 5 hr (see Fig. 3).

When sucrose was added to the mannose-treated slices considerable amounts of hexose appeared in the bathing solution (Table 3). During the 1st hr more glucose than fructose was released but by the end of the third hr more fructose than glucose was present. At the end of the first hr the decrease in sucrose was about equal to the increase in glucose.

TABLE 3. SUCROSE DISAPPEARANCE AND HEXOSE APPEARANCE IN SOLUTIONS BATHING MANNOSE-TREATED SLICES*

Time (hr)	Sucrose concn (mM)	Sucrose disappearance (μmol)	Hexose in bathing soln (μmol)		
			Glucose	Fructose	G/F
1	0	—	0·3	0·15	2·00
	2	3·9	4·1	2·8	1·46
	4	5·0	4·2	3·2	1·31
3	0	—	0·3	0·3	1·00
	2	12·4	4·0	6·0	0·66
	4	20·0	5·5	8·2	0·67
5	0	—	0·006	0·3	0·02
	2	17·5	1·2	5·1	0·24
	4	33·4	3·4	10·8	0·31

* Slices (1 g) were incubated in 10 ml of 0·1 M mannose at 30° for 1 hr. The mannose was then removed and the slices were rinsed in 10 ml of water and incubated in H_2O for 11 min. At the end of the short H_2O incubation the bathing solution was replaced with fresh water or sucrose solutions (time zero) and portions of the bathing solution were removed for sugar analysis at the times indicated above. These data are averages from the results of two experiments. Tissue sucrose (μmol): Time zero, 43; 5 hr in H_2O, 39; 5 hr in 2 mM sucrose, 46; 5 hr in 4 mM sucrose, 54.

[8] GARRARD, L. A. and HUMPHREYS, T. E. (1959) *Phytochemistry* **8**, 1065.

Thereafter, however, sucrose disappearance was correlated with sucrose concentration and, to some extent, with fructose appearance, but it bore no relation to glucose concentration.

TABLE 4. SUCROSE DISTRIBUTION AND SUGAR UPTAKE AND CATABOLISM IN SLICES INCUBATED IN SORBITOL AND SUGAR*

Incubation conditions	Total tissue	Sucrose (μmol/g fr. wt) Cytoplasmic	Stored	Sugar uptake (μmol/5 hr)	Sugar catabolized† (μmol hexose)
Sorbitol (1 M), 0·5 hr	58	15	43		
Sorbitol (1 M), 5 hr	57	15	42		
Sorbitol (0·9 M) + sucrose (0·1 M), 5 hr	122	44	78	113	96
Sorbitol (0·9 M) + glucose (0·1 M), 5 hr	118	43	75	212	90

* Fresh slices (1 g) were incubated in H_2O for 30 min at 30° after which they were placed in sorbitol or sorbitol plus sugar. Duplicate flasks were run for each treatment. At the end of the indicated length of time the slices from one flask of each set were killed, and the other slices were placed in H_2O to measure sucrose leakage (cytoplasmic sucrose).[1] The slices treated in sorbitol plus sugar were washed in sorbitol (1 M) and incubated in sorbitol for an additional 11 min following the 5 hr incubation in order to remove the free space sugars before killing the slices or transferring them to water. For sugar uptake measurements samples of the bathing solutions were taken 1 min and 5 hr after adding the sugar. The sorbitol-sucrose solution initially contained 0·7 mM glucose (present as an impurity in the sorbitol) and after 5 hr contained 3 mM glucose.

† The difference between sugar uptake and the increase in tissue sucrose after the 5 hr incubation calculated as hexose. The glucose content of the slices was in all less than 5 μmol/g.

The experiments on hexose release during sucrose uptake in DNP- and mannose-treated slices are interpreted as indicating that sucrose is split during transport. Experiments in which the amount and distribution of sucrose were measured following incubation of slices in sorbitol plus glucose or sucrose support this interpretation (Table 4). Sorbitol was used even though it inhibited sugar uptake about 50% because in 1 M sorbitol the slices were unable to utilize endogenous sucrose (thus simplifying interpretation of the results) and because sorbitol made it possible to estimate sucrose distribution within the cells.[1] The results obtained with glucose and sucrose were similar in all respects (Table 4). It is particularly noteworthy that the amounts of glucose taken up were very nearly twice those of sucrose or, on a carbon basis, equal amounts of the two sugars were taken up. This was found to be true at sugar concentrations of 0·05 M or above; at sugar concentrations below 0·05 M more carbon was taken up from sucrose than from glucose. In these experiments the total molarity of sugar plus sorbitol was 1·0 M, but similar results were obtained in the absence of sorbitol.[6]

DISCUSSION

During the rapid utilization of vacuolar sucrose in the presence of DNP significant quantities of hexose were not released into the bathing solution (Table 1, Fig. 2). These facts suggest (but are not sufficient to support a firm conclusion) that free hexose is not a product of sucrose transport at the tonoplast. The questions are, what would be the cytoplasmic concentrations of glucose and/or fructose necessary for a phosphorylation rate of 14 μmol/g/hr (a rate required by the data and calculations of Table 1), and would a measurable leakage of hexose occur at these concentrations.

Since DNP does not inhibit the penetration of hexose into the hexose space of the cells[5] and since in the presence of DNP the tissue hexose rapidly and almost completely leaked from the tissue (Fig. 1), it is assumed that slices placed in 2 mM hexose (Fig. 2) have a cytoplasmic hexose concentration of 2 mM. In Fig. 2, however, the rates of hexose uptake were much below those necessary to obtain the observed rates of vacuolar sucrose utilization. If the phosphorylation enzymes were saturated with hexose at very low hexose concentrations the additional 2 mM hexose would have little effect on the rate. This does not appear to be the case, for Cox and Dickinson[9] isolated a hexokinase from germinating maize scutella that had a K_m of 3·4 mM glucose and maximal velocity was reached at 40 to 50 mM glucose. Furthermore, scutellum slices placed in 10 mM glucose plus DNP took up glucose at about 15 μmol/g/hr;[6] a rate about 3-fold greater than that obtained in these studies with 2 mM glucose (Fig. 2). Note also that the rate of glucose uptake from about 1 mM glucose (Table 2) was about half the rate from 2 mM glucose (Fig. 2). Evidently, either the cytoplasmic hexose concentration (in the absence of added hexose) was greater than 2 mM or vacuolar sucrose utilization does not involve a free hexose intermediate. The latter suggestion appears to be more likely.

For sucrose transport at the plasmalemma it is concluded that sucrose is split into glucose and fructose during transport; that both hexoses remain bound within the transport apparatus; and that the bound hexoses are released into the cytoplasm only after being phosphorylated. These conclusions are supported by the following observations: (1) the appearance of glucose and fructose in the bathing solution during sucrose uptake into DNP- and mannose-treated slices (Fig. 3, Tables 2 and 3) and the equality of carbon uptake from glucose and sucrose (Table 4) indicate that sucrose is split during uptake; (2) extracellular hydrolysis of sucrose does not appear to be the source of hexose when mannose-treated slices were used because initially more glucose than fructose was released (Table 3) whereas when the two hexoses were added together they were taken up at equal rates; (3) the hexose could not have originated from cytoplasmic sucrose because sucrose is not the transport product[1] and because mannose-treated slices were unable to utilize (or hydrolyze) tissue sucrose (footnote, Table 3); (4) it also seems unlikely that the hexose in the bathing solution could arise from the action of cytoplasmic phosphatases on hexose phosphates, because the mannose-treated slices contained about 8 μmol of cytoplasmic M-6-P which remained untouched in the slices for at least 4 hr after removal of the mannose bathing solution;[4,10] (5) if free hexose were released into the cytoplasm as the final step in sucrose transport, a hexose concentration of 0·1 M would be required in the cytoplasm and also, from Fig. 1 and[5], in the bathing solution to account for the uptake rate in Table 4; only 3 mM glucose actually was found in the bathing solution (footnote, Table 4); (6) previous results,[6,11] obtained without DNP or mannose indicate that scutellum slices take up sucrose actively without releasing significant amounts of hexose; (7) if the glycosidic bond were utilized or preserved during sucrose uptake in the formation of UDPglucose and fructose or sucrose phosphate, it would be difficult to explain the equality in the rates of carbon uptake from sucrose and glucose solutions, except as fortuitous (Table 4).

A working model of the sucrose transport system of the plasmalemma is shown in Fig. 4. The model features a disaccharidase situated in the interior of the membrane and con-

[9] COX, E. L. and DICKINSON, D. B. (1973) *Plant Physiol.* **51**, 960.
[10] HUMPHREYS, T. E. (1972) *Phytochemistry* **11**, 541.
[11] HUMPHREYS, T. E. and GARRARD, L. A. (1968) *Phytochemistry* **7**, 701.

nected to the outer surface by a carrier and to the inner surface by a phosphate transferring enzyme. Sucrose is picked up by the carrier and passed on to the disaccharidase which, in turn, transports it to the phosphotransferase. When the sucrose reaches the phosphotransferase it is hydrolyzed and phosphorylated. The resulting hexose phosphates are released by the phosphotransferase into the cytoplasm.

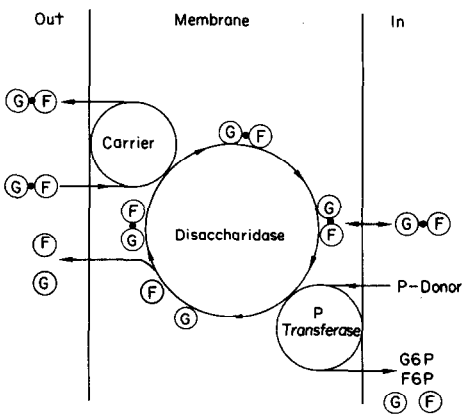

FIG. 4. A SPECULATIVE WORKING MODEL OF DISACCHARIDE MEMBRANE TRANSPORT. SEE TEXT FOR DETAILS.

The following points are pertinent: (1) the disaccharidase is shielded from water except near the phosphotransferase; (2) during transit a position is reached where the disaccharidase-bound sucrose can exchange with cytoplasmic sucrose. Sucrose molecules that are not hydrolyzed remain on the disaccharidase and are carried to the outer surface where they exchange with exogenous sucrose via the carrier. Exchange is slow because the disaccharidase is sequestered in the membrane. These features of the model account for the observed sucrose exchange,[11] and for the fact that cytoplasmic sucrose is utilized at the rate of vacuolar sucrose, not at the rate of exogenous sucrose;[1] (3) hexose molecules that are not phosphorylated are either released into the cytoplasm (whence they leak into the bathing solutions through hydrophilic pores in the membrane, see Fig. 1) or they remain on the disaccharidase and are carried towards the outer surface and are released. In the presence of DNP or mannose the level of phosphate donor decreases, and not all the hexose formed is phosphorylated. At the plasmalemma, the phosphate donor is assumed to be ATP; both mannose[8] and DNP would be expected to lower the ATP level; (4) in the presence of DNP, fructose is released in greater amounts than glucose (Fig. 3) because of its position on the disaccharidase (e.g. if the disaccharidase is an alpha glucosidase) or because of the specificity of the phosphotransferase; (5) sucrose and maltose are taken up by the corn scutellum at approximately the same rate. A disaccharidase which exhibits nearly the same V_{max} and K_m with sucrose as it does with maltose has been found in the scutellum (unpublished data). The enzyme is insoluble but not extracellular and is presumed to be in the membranes.

EXPERIMENTAL

Plant material. Maize grains (*Zea mays* L., cv. Funks G-76 or G-4455 were soaked in running tap water for 24 hr and then placed on moist paper towels in the dark at 24–25° for 72 hr. The scutella were excised and cut transversely into slices 0·5 mm or less in thickness. The slices were washed in H_2O until the washings remained clear, blotted on filter paper and weighed in groups of 1 g.

Analysis of sugars. The sucrose contents of the bathing solns and tissue extracts were determined by analyzing for glucose before and after invertase treatment. Glucose was determined by the glucose oxidase method. Fructose and glucose were assayed by converting them to hexose-6-P and then measuring the amount of $NADPH_2$ formed in the presence of G-6-P dehydrogenase (E.C. 1.1.1.49) as previously described.[+] Scutellum slices were killed in boiling 80% EtOH, and extracts for sugar analysis were prepared from the soln.[12] Methods for estimating the amounts of cytoplasmic and vacuolar sucrose have been described.[1]

Experimental procedure. All incubations except the cold treatment of Fig. 1 were carried out at 30° with flasks in a "gyrotory" water bath (New Brunswick Scientific Co., New Brunswick, N.J.) or, for gas exchange measurements, in a Warburg water bath.

Acknowledgement—I thank Dr. L. A. Garrard for helpful discussions.

[12] GARRARD, L. A. and HUMPHREYS, T. E. (1967) *Phytochemistry* **6**, 1085.