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SUCROSE TRANSPORT AT THE TONOPLAST*

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Key Word Index—*Zea mays*; Gramineae; maize scutellum; tonoplast; transport; sucrose storage; sucrose leakage.

Abstract—Sucrose that leaked from maize scutellum slices upon transfer of slices from a hexose or hexitol solution to water or upon placing the slices in a buffered EDTA solution was considered to be cytoplasmic in origin; residual (after leakage) tissue sucrose was considered to be stored in the vacuoles. This paper presents a study of the movement of sucrose across the tonoplast between the vacuoles and the cytoplasmic compartment. It is concluded that; (a) sucrose transport into the vacuoles is directly linked to sucrose synthesis in such a way that free sucrose is not an intermediate in the coupled process, (b) cytoplasmic sucrose is not (cannot be?) stored, (c) sucrose transport out of the vacuoles is linked to the metabolic demand for sugar, and (d) the transport process removing sucrose from the vacuoles does not release free sucrose into the cytoplasm. The sucrose fluxes at the plasmalemma and at the tonoplast are calculated, and the transport processes at the two membranes are compared.

INTRODUCTION

PREVIOUS work[1–3] indicates that cells of the maize scutellum contain two sucrose compartments: the cytoplasmic compartment and the storage compartment. The two-compartment idea is based on the observation that some of the sucrose in scutellum slices was leakable (cytoplasmic sucrose) and some was not (stored sucrose). It was concluded from this work that cytoplasmic sucrose does not leak through the plasmalemmas; rather it moves in the symplast to the phloem whence it leaks into the solution bathing the slices through the cut ends of the sieve tubes. Sucrose movement in the symplast is thought to occur in a pressure-flow of solution.[3,4] The leakage process is labile and scutellum slices incubated at 30° in water soon lose the ability to leak sucrose. However, leakage is maintained or restored by metal complexing agents such as EDTA.[2] High concentrations of hexose or hexitol inhibit sucrose leakage, but leakage resumes at an increased rate when the slices are removed from the hexose or hexitol solution and placed in water. Hexose and hexitol readily penetrate into the cytoplasm of these cells; therefore, when the slices are transferred to water the sudden increase in turgor pressure is thought to cause a rapid flow of solution in the symplast. It is estimated that more than 90% of the cytoplasmic sucrose is flushed from the tissue in this manner.[3] The sucrose flushed from the tissue is considered to be the sucrose that was in the cytoplasm at the time the slices were transferred to water; the sucrose remaining in the slices is considered to be stored sucrose. Because of the large amount of

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[1] GARRARD, L. A. and HUMPHREYS, T. E. (1971) *Phytochemistry* **10**, 243.
[2] HUMPHREYS, T. E. and GARRARD, L. A (1971) *Phytochemistry* **10**, 981.
[3] HUMPHREYS, T. E. and GARRARD, L. A. (1971) *Phytochemistry* **10**, 2891.
[4] HUMPHREYS, T. E. (1972) *Phytochemistry* **11**, 1311.

sucrose that is usually found in storage and because the vacuoles in these multivacuolate cells make up a large fraction of the water volume of the tissue, it is presumed that together the vacuoles make up the storage compartment of the cell.

This paper reports a study of the movement of sucrose into and out of the storage compartment, movement which would involve crossing the tonoplast.

RESULTS

Cytoplasmic Sucrose

The scutellum of the intact seedling immediately after removal of the endosperm contains a considerable amount of cytoplasmic sucrose which slowly leaks from the scutellum into water when the root–shoot axis is removed.[4] Recently, a much more rapid sucrose leakage into water has been obtained by placing the scutellum of an intact seedling in 0·5 M mannitol for 30 min before removing the axis. From the mannitol experiments it is estimated that half (50–60 μmol/g) of the scutellar sucrose was cytoplasmic at the time the endosperm was removed from 3-day-old seedlings. In the experiments reported herein scutellum slices were used, and much of the cytoplasmic sucrose leaked from the tissue during preparation of the slices. Therefore, 1 g of fresh slices contained only 20–25 μmol of cytoplasmic sucrose whereas the original 50–60 μmol of stored sucrose still remained.

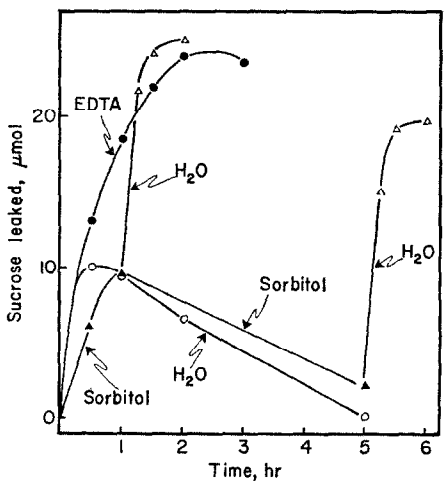

FIG. 1. CYTOPLASMIC SUCROSE IN FRESH SLICES.
Slices (1·0 g fr. wt) were placed in 10 ml of water, sorbitol (1·0 M) or EDTA (2·0 mM) plus glycylglycine buffer (0·06 M, pH 7·5) at zero time. Two groups of slices were placed in flasks with sorbitol; the sorbitol was replaced with water after 1 or 5 hr. Portions of the bathing solutions were removed for sucrose analysis at the times shown.

When fresh slices were placed in water, about 10 μmol of sucrose leaked during the first 30 min, and this sucrose was slowly reabsorbed (Fig. 1). A similar pattern of leakage and reabsorption occurred in 1·0 M sorbitol. The origin of this sucrose is not clear; it is different from the main pool of cytoplasmic sucrose because leakage occurred into 1·0 M sorbitol. Furthermore, the amount of sucrose appears to be too large (*ca.* 15% of the total tissue sucrose) for it to have come from cells injured during weighing the slices. In any case, this sucrose is counted as cytoplasmic sucrose and is found only in fresh slices.

When slices were transferred from 1·0 M sorbitol to water, an additional 15–16 μmol of cytoplasmic sucrose was flushed from the tissue (Fig. 1). When fresh slices were placed directly into a buffered EDTA solution the amount of sucrose leakage was about equal to the total leakage obtained in the sorbitol–water incubation sequence (Fig. 1). Apparently, EDTA reduced the resistance to leakage whereas transfer from sorbitol to water increased the driving force and under either condition the leakage pathway was open.

About the same amount of sucrose leaked into water after 5 hr in sorbitol as after 1 hr in sorbitol (Fig. 1) indicating that the slices cannot utilize cytoplasmic sucrose during incubation in 1·0 M sorbitol.

Sucrose Transport into the Vacuoles

When fresh slices are placed in fructose solutions there is a rapid synthesis of sucrose. Previous work[4,5] shows that in 1·0 M fructose much of the newly synthesized sucrose remains in the cytoplasm (from where it leaks when the slices are transferred to water), whereas most of the sucrose synthesized in 0·1 M fructose is presumed to be stored in the vacuoles (since very little sucrose leaks when these slices are transferred to water). However, the turgor pressure developed upon transfer of slices from 0·1 M fructose to water may not overcome the resistance to leaking, and sucrose which appeared to be stored may instead be trapped in the cytoplasm.

An experiment using EDTA to unblock the leakage pathway confirmed the previous conclusion that most of the sucrose synthesized in 0·1 M fructose is stored. In this experiment slices were incubated for 3·5 hr in 0·1 M fructose or water and then were placed (time zero in Fig. 2) in EDTA or EDTA plus 0·1 M fructose. The fresh slices contained 86 μmol of of sucrose per g, of which about 10 μmol readily leaked from the slices, about 15 μmol were cytoplasmic and required EDTA or high turgor pressure before they leaked and the remaining 61 μmol were in storage (see Fig. 1). After 3·5 hr in fructose the slices contained 153 μmol of sucrose per g of which 14 μmol were cytoplasmic (i.e. they leaked into EDTA, Fig. 2, curve 2) and 139 μmol were in storage. Therefore, during 3·5 hr in 0·1 M fructose the slices stored 78 μmol of sucrose (139 minus 61) or 22 μmol per g hr.

When slices which had been incubated in 0·1 M fructose for 3·5 hr were placed in 0·1 M fructose plus EDTA, sucrose synthesis continued but at a much lower rate and sucrose leakage and synthesis went on at about the same rate (Fig. 2, curve 1). Little, if any, storage of sucrose occurred during incubation in fructose plus EDTA.

In contrast, slices incubated in water for 3·5 hr contained only 58 μmol sucrose per g; a decline of 28 μmol of which about 3 μmol remained in the water (see Fig. 1) and 25 μmol were metabolized. The sucrose remaining in the slices was almost entirely in the vacuoles since only about 1 μmol leaked into EDTA (Fig. 2, curve 4). When the slices were placed in EDTA plus fructose, a rapid sucrose synthesis occurred; in 3 hr the tissue level increased to 128 μmol and 8 μmol leaked into the bathing solution (Fig. 2, curve 3). Since the leakage pathway was open, the increase in tissue sucrose represents stored sucrose; a storage rate of 23 μmol per g hr.

This experiment shows that sucrose synthesis and sucrose storage are controlled in some manner by the sucrose concentration of the vacuoles, and the cytoplasmic sucrose concentration does not appear to influence the two processes. The latter conclusion is supported by the experiments of Fig. 3 and Table 1 which indicate that cytoplasmic sucrose is not involved in sucrose storage. Rather, they indicate that storage is linked to synthesis and free

[5] HUMPHREYS, T. E. and GARRARD, L. A. (1966) *Phytochemistry* **5**, 653.

sucrose is not an intermediate in the coupled process. Fresh slices were incubated in 1·0 M fructose for 3 hr to increase the amount of cytoplasmic sucrose. One group of slices was then transferred to water where the resulting sudden increase in turgor pressure flushed the cytoplasmic sucrose (*ca.* 41 μmol) into the bathing solution (Fig. 3, curve 1). The other groups of slices were transferred to a $CaCl_2$ solution which inhibited sucrose leakage[6] but allowed fructose to leave the cells (unpublished results). Slices were transferred to EDTA after 15, 60 and 150 min in $CaCl_2$ (Fig. 3, curves 2,3,6), and the subsequent leakage shows

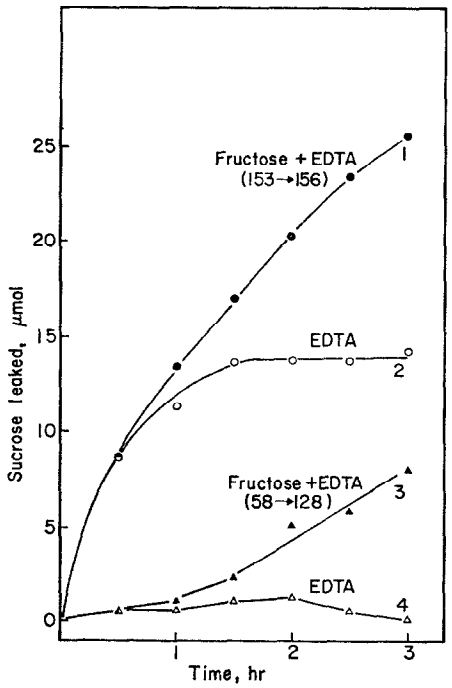

FIG. 2. CYTOPLASMIC AND STORED SUCROSE CONTENT OF SLICES AFTER INCUBATION IN WATER OR FRUCTOSE. Slices (1·0 g fr. wt) were incubated (in triplicate) in water or fructose (0·1 M) at 30° for 3·5 hr, after which one group from each set of three was killed to obtain total tissue sucrose and the remaining groups from each set were transferred to two buffered EDTA solutions (see legend, Fig. 1) one of which also contained fructose (0·1 M). The solution volume was 10 ml After 3 hr in fructose plus EDTA the slices were killed to obtain total tissue sucrose. Portions of the bathing solution were removed at the times shown for sucrose analysis. Curves 1 and 2; leakage from slices which had been incubated in fructose for 3 5 hr. Curves 3 and 4; leakage from slices given a prior 3 5-hr incubation in water. The numbers in brackets refer to the sucrose content (μmol/g) of the slices at zero time in the graph (first number) and after 3 hr in EDTA plus fructose (second number).

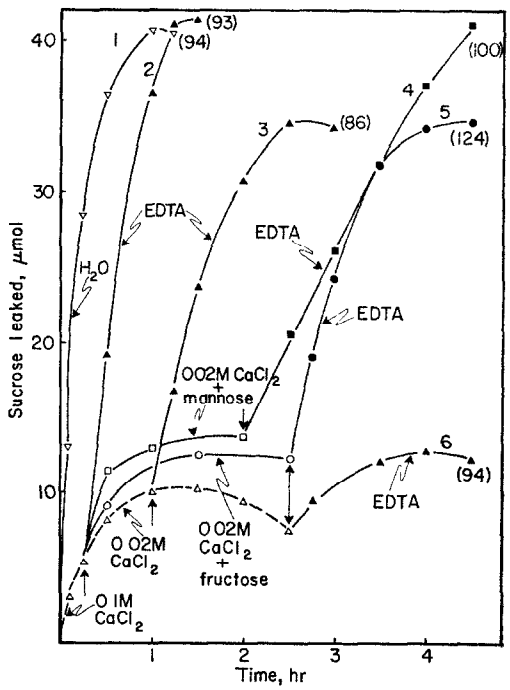

FIG. 3. DECLINE IN THE AMOUNT OF CYTOPLASMIC SUCROSE DURING INCUBATION OF SLICES IN $CaCl_2$. Slices (1·0 g fr wt) were incubated in 1·0 M fructose at 30° for 3 hr One group of slices was then quickly rinsed with water and placed in 10 ml of H_2O (curve 1) After 5 min a portion of the bathing solution was removed for sucrose analysis, and the bathing solution was replaced with fresh H_2O; this step removed most of the free space fructose The other five groups of slices were each rinsed with $CaCl_2$ (0·1 M) and placed in 10 ml of $CaCl_2$ (0 1 M). After 15 min the $CaCl_2$ bathing solution was replaced with a buffered EDTA solution (curve 2), 0 02 M $CaCl_2$ (curves 3 and 6), 0·02 M $CaCl_2$ plus 0 05 M mannose (curve 4), or 0·02 M $CACl_2$ plus 0·1 M fructose (curve 5) These solutions, in turn, were replaced by a buffered EDTA solution (see legend, Fig. 1) as the times indicated by the straight arrows. The numbers in brackets are the amounts of tissue sucrose (μmol) at the end of the leakage period.

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

the decline in cytoplasmic sucrose with length of time in $CaCl_2$. Although the amount of cytoplasmic sucrose declined rapidly during incubation in $CaCl_2$, the amount of stored sucrose (tissue sucrose at the end of the leakage periods given by the numbers in brackets, Fig. 3) stayed at about the same level as that of the water control.

TABLE 1. FATE OF CYTOPLASMIC SUCROSE PREVENTED FROM LEAKING BY $CaCl_2$*

Bathing solution during leakage period	Tissue level after leakage (stored)	Sucrose† μmol/g Leaked (cytoplasmic)	Consumed (estimated)	Total
H_2O (1·5 hr)	98 ± 5 (5)	39 ± 1 (5)	9	146
Ca^{2+} (0·25 hr), EDTA (1·25 hr)	94 ± 1 (3)	41 ± 3 (3)	9	144
Ca^{2+} (2·5 hr), EDTA (2 hr)	97 ± 4 (3)	14 ± 2 (3)	27	138

* Slices (1·0 g fr. wt) were incubated in 1·0 M fructose at 30° for 3 hr and then transferred to water or $CaCl_2$. See Fig. 3 for details.

† The values given are averages followed by s.d. and the number of experiments in brackets. The amount of sucrose consumed during the leakage period was estimated assuming the same rate of consumption found with fresh slices in water (6·0 μmol/g hr, Table 2). At the start of the leakage period, the slices contained 138 ± 3 (5) μmol/g.

Table 1 is a balance sheet compiled from a number of experiments like that of Fig. 3 showing sucrose distribution after leakage into water or into $CaCl_2$ followed by EDTA. The amount of stored sucrose did not change during a 4·5-hr incubation in $CaCl_2$–EDTA and it is concluded that the decline in cytoplasmic sucrose was a result of metabolic utilization, not storage.

When fructose was present with the $CaCl_2$ solution during a 2·5 hr incubation (Fig. 3, curve 5) the amount of cytoplasmic sucrose remained at a high level (compare curves 5 and 6), and the amount of stored sucrose increased to 124 μmol. Apparently fructose was preferentially used in metabolism sparing the cytoplasmic sucrose (see Fig. 2, curve 2), and an additional 30 μmol of sucrose newly synthesized from fructose were stored. It is concluded from this that the sucrose storage mechanism was functional in these slices and that storage is directly linked to synthesis.

When mannose, which greatly inhibited the metabolic utilization of endogenous sucrose (Table 2) was added to the $CaCl_2$ solution (Fig. 3, curve 4) the amount of cytoplasmic sucrose at the end of a 2-hr incubation was at least as high as that found in the water control (curve 1). This experiment shows that EDTA opened the leakage channels closed by $CaCl_2$; and, therefore, the amounts of leakage shown in Fig. 3 correctly represents amount of cytoplasmic sucrose in the slices.

Sucrose Transport out of the Vacuoles

In Table 2 the rates of metabolic utilization of sucrose are given for slices incubated in water, in sorbitol and mannose (which inhibited utilization), and in DNP (which increased utilization).

When fresh slices are placed in water there are three sources of sucrose for metabolism: about 10 μmol of exogenous sucrose, a result of the initial leakage, which is slowly taken up

by the slices (see Fig. 1), about 15 μmol of cytoplasmic sucrose, and stored sucrose. Nevertheless, the utilization rate in water given in Table 2 and the maximum rate (under the experimental conditions) of sucrose transport out of the vacuoles are probably equal. This conclusion is based on the observation that in the absence of both exogenous and cytoplasmic sucrose (as a result of removal and depletion, respectively) the rate of sucrose utilization remained unchanged (5–6 μmol/g hr).

TABLE 2. UTILIZATION OF ENDOGENOUS SUCROSE*

Bathing solution	Change in sucrose content (slices + bathing solution) μmol/g hr	Bathing solution	Change in sucrose content (slices + bathing solution) μmol/g hr
H_2O	−6·0	Mannose (0·1 M) + sorbitol (0·9 M)	+0·7
Sorbitol (1·0 M)	−2·0	DNP (5×10^{-4} M)	−11·3
Mannose (0·1 M)	−1·5	DNP (5×10^{-4} M) + sorbitol (1·0 M)	−7·2

* Slices (1·0 g fr. wt) were incubated for 5 hr in 10 ml of the above bathing solutions. Samples of the bathing solutions were taken for sucrose analysis and the slices were killed. The values given are averages of the results of three experiments.

Slices placed in 1·0 M sorbitol utilized sucrose at a rate of only 2·0 μmol/hr g (Table 2); however, as shown in Fig. 1, this is the rate at which sucrose which initially leaked into the sorbitol bathing solution was reabsorbed. In another experiment, when the exogenous sucrose source was removed by replacing the bathing solution with fresh sorbitol at the end of the first hr, sucrose utilization stopped and the amount of tissue sucrose (15 μmol cytoplasmic, 49 μmol stored) remained constant for at least 4 hr. Therefore, 1·0 M sorbitol prevents the utilization of cytoplasmic sucrose and the transport and utilization of stored sucrose, but allows the transport and utilization of exogenous sucrose.

Mannose also strongly inhibited sucrose utilization (Table 2). When mannose was present, the sucrose that initially leaked from the slices was not reabsorbed.

DNP caused almost a doubling of the rate of sucrose utilization compared with the water control (Table 2). DNP also allowed the slices to utilize endogenous sucrose in the presence of 1·0 M sorbitol. Indeed, the sucrose utilization rate in DNP plus sorbitol was greater than that in water.

Table 3 shows the distribution of sucrose between cytoplasm and vacuole after 5-hr incubations in sorbitol and sorbitol plus mannose or DNP. After 5 hr in sorbitol alone the amount and distribution of sucrose in the slices was the same as it was at zero time (with the exception that fresh slices contained 11 μmol of sucrose that leaked during the first hr and were reabsorbed and utilized during the next 4 hr (see Fig. 1 and Table 2). In DNP plus sorbitol the amount of cytoplasmic sucrose remained the same as that of the sorbitol control even though the vacuolar pool was being rapidly depleted. In other experiments, longer (8-hr) incubation periods in DNP or DNP plus sorbitol were used. In DNP alone the tissue sucrose decreased to less than 5 μmol/g; in DNP plus sorbitol the tissue sucrose decreased to 19 μmol/g, of which 15 were cytoplasmic and less than 5 were vacuolar. Therefore, it appears that cytoplasmic sucrose cannot be utilized in the presence of DNP and sorbitol.

Sucrose utilization completely stopped in mannose plus sorbitol, but transport (leakage?) of sucrose out of storage occurred (Table 3). In a separate experiment, the amount of cytoplasmic sucrose was measured after 2, 4 and 6 hr in sorbitol (0·9 M) plus mannose (0·1 M) and was found to steadily increase at a rate of 3–4 μmol/hr.

TABLE 3. SUCROSE DISTRIBUTION FOLLOWING INCUBATION OF SLICES IN SORBITOL PLUS DNP OR MANNOSE*

Bathing solution	Sucrose (μmol/g) at end of incubation		
	Cytoplasmic	Stored	Total tissue
Sorbitol (1·0 M)	16	56	72
Sorbitol (1·0 M) + DNP (5×10^{-4} M)	15	16	31
Sorbitol (0·9 M) + mannose (0·1 M)	32	40	72

* Slices (duplicate groups of 1·0 g fr. wt each) were incubated for 5 hr at 30°. At the end of the period one group from each set was killed to obtain the total tissue sucrose. The bathing solution was removed from the other group of each set, the slices were rinsed with 10 ml of water and 10 ml of water were added. These slices were then incubated at 30° for an additional 90 min, and the amount of sucrose that leaked was the cytoplasmic sucrose. The amount of stored sucrose was obtained by difference. The fresh slices at the start of the 5 hr incubation contained 83 μmol/g.

DISCUSSION

The following conclusions are drawn: (a) sucrose transport into the vacuoles is directly linked to sucrose synthesis in such a way that free sucrose is not an intermediate in the coupled process, (b) cytoplasmic sucrose is not (cannot be?) stored, (c) sucrose transport out of the vacuoles is linked to the metabolic demand for sugar, and (d) the transport process removing sucrose from the vacuoles does not release free sucrose into the cytoplasm.

It appears that there is no net transport in either direction between the cytoplasmic and vacuolar sucrose pools. They may be linked indirectly, of course, through hexose or hexose derivatives such as nucleotide sugars and hexose phosphates. The evidence is quite good (Fig. 3, Table 1) that cytoplasmic sucrose is not transported into the vacuole; the idea that transport out of the vacuole does not result in the release of free sucrose into the cytoplasm is based on the evidence that, in the presence of DNP plus sorbitol, vacuolar sucrose but not cytoplasmic sucrose can be utilized.

Garrard and Humphreys[6,7] concluded that cytoplasmic sucrose could be stored during $CaCl_2$ inhibition of sucrose leakage, and that newly synthesized sucrose (but not exogenous sucrose) could be stored in the presence of mannose. However, at that time it was not known that sucrose leakage was a labile process; therefore, sucrose was considered to be stored if it did not leak into water.

The idea that sucrose synthesis and sucrose storage are coupled processes was suggested by Glasziou[8] and by Sacher *et al.*[9] Hatch[10] presented evidence that sucrose phosphate is involved in sucrose accumulation in sugar cane. He proposed that sucrose synthesis and storage are linked through sucrose phosphate which functions both as the transport form of sucrose and as an intermediate in sucrose synthesis. Subsequently, Hawker and Hatch[11]

[7] GARRARD, L. A. and HUMPHREYS, T. E. (1969) *Phytochemistry* **8**, 1065.
[8] GLASZIOU, K. T. (1961) *Plant Physiol.* **36**, 175.
[9] SACHER, J. A., HATCH, M. D. and GLASZIOU, K. T. (1963) *Plant Physiol.* **38**, 348.
[10] HATCH, M. D. (1964) *Biochem. J.* **93**, 521.
[11] HAWKER, J. S. and HATCH, M. D. (1966) *Biochem. J.* **99**, 102.

described a specific sucrose phosphate phosphatase from sugarcane which could catalyze the final reaction in the transport-accumulation process. Since the activities of sucrose phosphate synthetase (UDP-glucose: fructose-2-glucosyl transferase, E.C. 2.4.1.14) and sucrose phosphate phosphatase in the maize scutellum appear to be sufficient to account for the observed rates of sucrose synthesis and storage,[12] the above scheme would accomodate the results of this paper (Figs. 2 and 3).

The mechanisms of sucrose transport into the cytoplasm from the plasmalemma and the tonoplast appear to be different. Sorbitol completely inhibited movement across the tonoplast but not across the plasmalemma (Fig. 1, Table 2). Furthermore, DNP caused a doubling in the rate of sucrose transport out of the vacuoles (Table 2) but almost totally inhibited sucrose uptake across the plasmalemma.[13] A point of similarity is that the transport mechanism in neither membrane appears to release free sucrose into the cytoplasm. This is evidenced by the inability of the slices in sorbitol to utilize cytoplasmic sucrose whereas they transported and utilized exogenous sucrose (Fig. 1, Table 2), and by the ability of the slices to utilize stored sucrose but not cytoplasmic sucrose when incubated in sorbitol plus DNP (Table 3).

Estimates of the sucrose fluxes at the tonoplast and plasmalemma can be made from the results of this and a previous paper.[13] The average diameter of a scutellum cell is estimated to be (from electron micrographs) 50 μm, and 80% of the fr. wt of the slices is considered to be contained within the plasmalemmas. Therefore, one g fr. wt of slices would contain about 10^3 cm^2 of plasmalemma surface. Because the scutellum cells are multivacuolate, estimates of total tonoplast surface are subject to considerable error. Estimates are likely to be too small because of the presence of many very small vacuoles not taken into account in deriving an average vacuolar diameter. The average diameter of the vacuoles is estimated to be 5 μm, and 70% of the water volume of the tissue (which is about 80% water) is considered to be in the vacuoles. Therefore, one g fr. wt of slices would contain about 7×10^3 cm^2 of tonoplast surface.

It is of interest to compare the two sucrose fluxes into the cytoplasm, one through the tonoplast and the other through the plasmalemma. Through the tonoplast the flux ranges from 0·23 to 0·45 pmol/cm^2 sec (for slices in water or DNP, Table 2); through the plasmalemma the maximum flux is 12·3 $p$mol/cm^2 sec (V_{max} of 45 μmol/g hr, Ref. 13). Sucrose uptake at the plasmalemma has a K_m of 20 mM (ref. 13). The flux at the tonoplast takes place from an initial vacuolar sucrose concentration of about 0·1 M. This flux remains constant as the vacuolar sucrose content is reduced; however, it is not known to what extent the vacuoles shrink as sucrose is removed. It was concluded above that sucrose uptake from the vacuoles is controlled by the metabolic demand for sugar; sucrose uptake across the plasmalemma is controlled in some other way.[13,14]

The sucrose flux across the plasmalemma of the scutellum is higher by a factor of about 250 than that found in carrot calluses,[15] and also is higher than inorganic ion fluxes of most plant cells.[16] That the scutellum plasmalemma should have such a high capacity for sucrose transport is puzzling. Sucrose transport within the scutellum appears to be symplastic,[3,4] and there is no evidence that secretion and uptake across the plasmalemmas are involved. Furthermore, in the intact seed the sugars presented to the scutellum are breakdown pro-

[12] HAWKER, J. S. (1971) *Phytochemistry* **10**, 2313.
[13] WHITESELL, J. H and HUMPHREYS, T E (1972) *Phytochemistry* **11**, 2139.
[14] HUMPHREYS, T. E. and GARRARD, L. A. (1968) *Phytochemistry* **7**, 701.
[15] EDELMAN, J. and SCHOOLAR, A. I. (1971) *J. Exp. Botany* **22**, 534.
[16] MACROBBIE, E. A. C. (1970) *Q. Rev Biophys.* **3**, 251.

ducts of starch mainly glucose and maltose. It may be that the transport mechanism of the plasmalemma, which utilizes sucrose in these experiments, functions in the intact seedling as a maltose transporter. Maltose is taken up by scutellum slices at about the same rate as sucrose.[13]

EXPERIMENTAL

Plant material. Maize grains (*Zea mays* L., cv. Funks G-76) were soaked in running tap water for 24 hr and then placed on moist paper towels in the dark at 24–5° 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 1·0 g.

Analysis of sucrose. Sucrose was determined by the glucose oxidase method (Glucostat, Worthington Biochemical Corp. Freehold, N.J.). Samples were incubated for 2 hr with and without invertase prior to analysis. Scutellum slices were killed in boiling 80% EtOH. The extraction and analysis of tissue sucrose has been described.[6]

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