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SUCROSE LEAKAGE FROM THE MAIZE SCUTELLUM*

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Abstract—Sucrose accumulated in the cytoplasm of mesophyll, parenchyma cells when maize scutella (whole or sliced) were put in concentrated (e.g. 1 0 M) fructose solutions. This accumulated cytoplasmic sucrose leaked from the tissue when the fructose solution was replaced with water or with a more dilute hexitol solution. The amount of leakage was proportional to the concentration difference between the fructose solution bathing the scutellum slices during the sucrose accumulation period and the hexitol solution bathing the slices during the leakage period. Only small amounts of cytoplasmic sucrose leaked from the whole scutellum into water until the root–shoot axis was removed. Other substances also leaked, with sucrose, from the scutellum. Sucrose, nitrogenous compounds, K^+ and phosphorous compounds leaked in greatest amounts. The results presented are consistent with the ideas of the mass flow hypothesis. In the scutellum system a pressure flow of solution originates in the mesophyll cells, flows from cell to cell through plasmodesmata, into and through the phloem sieve tubes, and, finally, into the bathing solution.

INTRODUCTION

IN PREVIOUS papers,[1,2] we proposed that in the excised, whole, maize scutellum or in scutellum slices sucrose leakage originates in the phloem and is the result of a series of events starting with sucrose synthesis and is dependent upon intercellular transport, vein loading and phloem transport of sucrose. The results of these studies are consistent with the idea that there exists in the scutellum a pool of leakable sucrose (probably located in the cells of the vascular tissue) from which sucrose is loaded into the sieve tubes and to which sucrose is transported from the mesophyll cells. It appears[1,2] that sucrose stored in the vacuoles of the mesophyll cells is only slowly made available for transport whereas newly synthesized sucrose of the cytoplasmic synthesis compartments of these cells[3,4] is rapidly transported to the pool.

The results presented in this paper indicate that a mass flow (pressure flow) of solution can occur from the mesophyll cells of the scutellum through the vascular tissue and into the bathing solution.

RESULTS

Accumulation of Sucrose in the Cellular Synthesis Compartment

When scutellum slices are placed in a 0·1 M fructose solution they synthesize sucrose (*ca.* 25 μmole/hr g fresh wt.) and some of the sucrose leaks into the bathing solution.[3] When these slices are removed from the fructose solution, washed briefly and then placed in

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[1] L. A. GARRARD and T. E. HUMPHREYS, *Phytochem.* **10,** 243 (1971).
[2] T. E. HUMPHREYS and L. A. GARRARD, *Phytochem.* **10,** 981 (1971).
[3] T. E. HUMPHREYS and L. A. GARRARD, *Phytochem.* **5,** 653 (1966).
[4] L. A. GARRARD and T. E. HUMPHREYS, *Phytochem.* **8,** 1065 (1969).

water there is little or no additional leakage. The newly synthesized sucrose that does not leak is assumed to have been transported into the vacuoles.[3,4] When slices are placed in more concentrated fructose solutions, however, sucrose transport is inhibited and some of the newly synthesized sucrose remains in the synthesis compartments of the cells. When these slices are removed from the fructose solution and placed in water there occurs a rapid leakage of the accumulated sucrose.[3] The scutellum is largely composed of mesophyll, parenchyma cells and, presumably, it is in these cells that most of the sucrose synthesis and accumulation occurs.

The results of a typical experiment during which this leakage of accumulated sucrose was determined are shown in Fig. 1. The slices were incubated for 3 hr in 1·0 M fructose during which period they synthesized about 80 μmoles of sucrose (approximately doubling their sucrose content) and leaked about 15 μmoles of sucrose into the fructose bathing solution. The slices were then placed in water or in 1·0 M sorbitol In contrast to a rapid sucrose leakage into water, there was a slow leakage into sorbitol. However, when the sorbitol was replaced with water, sucrose leaked at a rate equal to the rate of leakage from

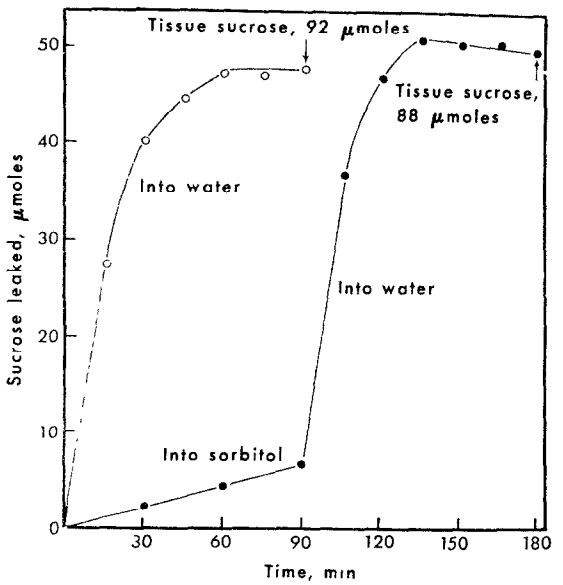

FIG. 1. LEAKAGE OF ACCUMULATED SUCROSE OF THE SYNTHESIS COMPARTMENTS OF CELLS OF SCUTELLUM SLICES.

The slices (1·0 g fresh wt.) were incubated in 1·0 M fructose for 3 hr at 30°. At the end of this period, the bathing solution was removed and the slices washed by rapid addition and removal of 10 ml of water or 1·0 M sorbitol. Then 10 ml of water or sorbitol were added (time zero on the graph) and after 90 min the sorbitol was replaced with water. Portions of the bathing solution were removed for sucrose analysis at the times shown. At the end of the period the slices were extracted with ethanol to determine the tissue sucrose.

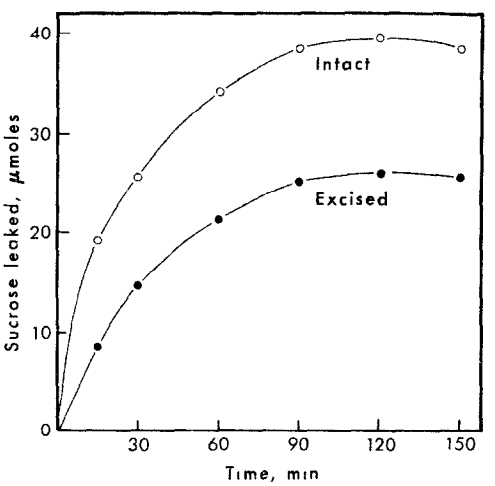

FIG. 2. LEAKAGE OF SUCROSE FROM WHOLE SCUTELLA. Endosperms were carefully removed and one group of 12 scutella (the intact group) with their root–shoot axes still attached were treated with 1·0 M fructose as described in Table 1. Another group of 12 seedlings (the excised group) had their root–shoot axes removed (by making a single cut at the node) and the excised scutella were placed in a flask with 10 ml of 1·0 M fructose. Both groups were incubated with 1 0 M fructose for 3 hr, but the 'intact' group was incubated at room temperature (*ca.* 23°) whereas the 'excised' group was incubated at 30°. At the end of the fructose incubation, the root–shoot axes were removed from the 'intact' group and the scutella were placed in water at 30°; the 'excised' group were also transferred to water at 30°. The start of the water incubation corresponds to time zero on the graph. Each group of 12 scutella weighed about 1 2 g.

slices which were transferred directly from fructose to water. High concentrations of both fructose and sorbitol inhibited sucrose transport into the vacuoles (storage) and out of the cells (leakage); therefore, sucrose accumulated (slices in fructose) or remained (slices in sorbitol) in the synthesis compartment of the cell. During the 90 min period that sucrose was held in the slices by 1·0 M sorbitol it was not degraded to hexose or otherwise metabolized, and previous work[4] indicates that this sucrose is in the cell as free sucrose.

In this paper we have investigated the leakage pathway and the driving force causing accumulated sucrose of the synthesis compartments to leak from the tissue. Experiments with the whole scutellum provided evidence for the leakage pathway.

Sucrose Leakage from the Whole Scutellum

It was also possible to demonstrate sucrose accumulation and leakage using whole scutella (Fig. 2). In these experiments, the root–shoot axes were removed from scutella either just before the fructose incubation or at the end of this incubation and just before transferring the scutella to water. The greatest amount of leakage occurred in the latter case in which the root–shoot axes were attached during the sucrose accumulation period. In both cases, however, the leakage was essentially complete after 90 min. The rate of leakage and the total amount leaked was less with whole scutella than with slices (compare Fig. 1 with lower curve, Fig. 2).

Removal of the root–shoot axis was necessary for an appreciable amount of sucrose leakage to occur, as is shown from experiments in which leakages from individual scutella were determined (Table 1). The scutella, with root–shoot axes attached, were placed in

TABLE 1. EFFECT OF THE PRESENCE OF THE ROOT–SHOOT AXIS ON SUCROSE LEAKAGE FROM THE SCUTELLUM*

Condition of scutellum during leakage	Sucrose leaked† μmoles/scutellum
Root–shoot attached	0·75 ± 0·5
Root–shoot excised	4·12 ± 0·9

* The endosperm was carefully removed from the seedlings. The seedlings were placed in ice water as they were prepared and were rinsed three times in ice water before use. After blotting on filter paper, each seedling was placed in a plastic, round-bottomed, 50-ml, centrifuge tube which had been cut down to about one-third its original height. The seedling was placed so that the scutellum (abaxial surface down) rested on the bottom and the primary root and the shoot were bent upward along the wall of the tube. Enough fructose solution (1·0 M) was then added to reach but not cover the scutellar node. In some cases the fructose solution covered all or part of the lateral seminal roots (which arise just above the node) depending on their length and position in the tube. The tubes were placed in a specimen jar which was lined with moist paper and covered with aluminium foil. The jar was kept at room temp. (*ca.* 23°) for 3 hr. At the end of this period the seedlings were removed from the fructose solution, quickly rinsed with water, blotted and then either replaced intact in tubes containing 1·0 ml of water or the root–shoot axes were removed (by making a single cut at the node) and the scutella were placed in tubes containing 1·0 ml of water. The tubes were replaced in the specimen jar and sucrose leakage was allowed to proceed for 90 min. At the end of this period portions of the solution were taken for analysis and the scutella were collected and weighed individually.

† Each number is the average of the sucrose leaked from the scutella of 24 seedlings (four experiments) followed by the standard deviation. Each scutellum weighed about 0·1 g.

1·0 M fructose and allowed to accumulate sucrose for 3 hr; then the scutella were placed in water with the root–shoot axes attached or removed. The removal of the root–shoot axis resulted in approximately a five-fold increase in leakage (Table 1). Leakage was more variable when the root–shoot axes were attached and in some experiments there were a few seedlings from which no leakage could be detected. Therefore, at least part of the sucrose leakage from the intact seedling probably resulted from injury to the root or shoot due to handling or to the high fructose concentration. The lateral seminal roots are most likely to have been the leakage source since they were fragile and were often in contact with the fructose solution. The relatively high amounts of glucose before adding invertase is further evidence for sugar leakage from the root and shoot since these tissues have high levels of glucose (*ca.* 60 μmoles/g) whereas the scutellum has only about 2 μmoles of glucose/g fresh wt.[5]

TABLE 2. EFFECT OF A WATER INCUBATION BEFORE REMOVAL OF THE ROOT–SHOOT AXIS ON SUBSEQUENT SUCROSE LEAKAGE*

Treatment sequence following 3 hr in 1·0 M fructose	Sucrose leaked† μmoles/scutellum
90 min in water, root–shoot axis attached, followed by 90 min in water, root–shoot axis removed.	0·73 ± 0·5
	2·18 ± 0·7

* See footnote, Table 1.

† Each number is the average of sucrose leakage from the scutella of 12 seedlings followed by the standard deviation.

Removal of the root–shoot axis at the end of the 90 min leakage period in water resulted in an additional sucrose leakage (Table 2). The sum of the two leakages (leakage into water before and after removal of the root–shoot axis) is about 70 per cent of that obtained when the axis was removed at the end of the fructose incubation (Table 1). During the first water incubation when the presence of the root–shoot axis prevented leakage, part of the accumulated sucrose in the cellular synthesis compartments may have been transported into storage (vacuoles) within the scutellum or transported to the root and shoot. Another possible explanation of these results (Table 2) is that during the initial water incubation (root–shoot axis attached) fructose leaked from the cells lowering the osmotic potential of the cytoplasm, thus decreasing the driving force responsible for the mass flow of solution out of the tissue. Evidence for a mass flow is given in the next section.

Mass Flow of Solution During Sucrose Leakage

Scutellum slices were placed in fructose so that sucrose would accumulate in the cytoplasmic synthesis compartments. After a period of accumulation, the slices were transferred to water or to sugar alcohol solutions and the amount of sucrose leakage was determined (Figs. 3 and 4). The amount of sucrose that leaked was directly proportional to the concentration difference between the fructose solution bathing the slices during the sucrose

[5] T. E. HUMPHREYS and L. A. GARRARD, *Phytochem.* 3, 647 (1964).

accumulation period and the hexitol (mannitol or sorbitol) solution bathing the slices during the leakage period. Thus, in the experiment of Fig. 3, about the same amount of leakage was obtained after transferring the slices from 0·9 M fructose to 0·3 M mannitol as was obtained after the transfer from 0·6 M fructose to water. Similarly, the transfers from 0·6 M fructose to 0·3 M mannitol and from 0·9 M fructose to 0·6 M mannitol resulted in about the same leakage. The greatest amount of leakage occurred when the slices were transferred from 0·9 M fructose to water, whereas concentration differences between the

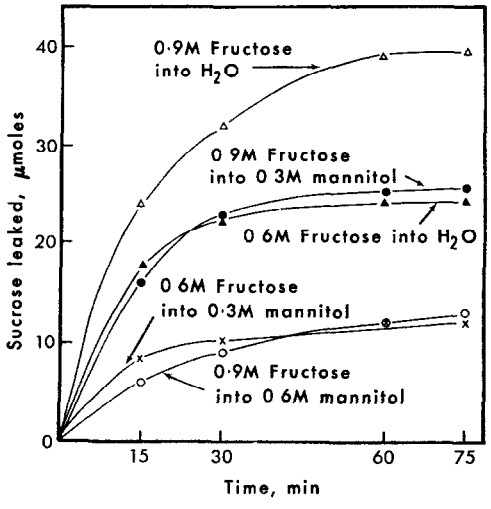

FIG. 3. THE EFFECT OF BATHING SOLUTION CONCENTRATION ON THE LEAKAGE OF SUCROSE.

The slices (1·0 g fresh wt.) were incubated in fructose (at the concentrations shown) for 3 hr at 30°. At the end of this period, the slices were put in water or mannitol (time zero on graph) following the procedures described in Fig. 1.

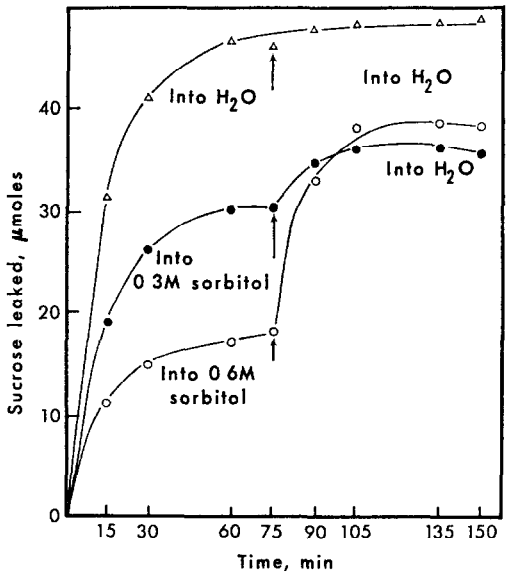

FIG. 4. STEPWISE RELEASE OF SUCROSE FROM THE CYTOPLASMIC SYNTHESIS COMPARTMENTS.

The slices (1·0 g fresh wt.) were incubated in 0·9 M fructose for 3 hr at 30°. At the end of this period they were transferred to water or to sorbitol (time zero on graph). After 75 min the sorbitol was replaced with water.

initial and final bathing solutions of 0·3 M and 0·6 M resulted in one-third and two-thirds of that amount of leakage, respectively. Essentially the same results were obtained in the experiment of Fig. 4. In this experiment the slices were incubated in 0·9 M fructose and were then transferred to water or to sorbitol where they remained for 75 min. At the end of this period the slices were transferred to water (arrows, Fig. 4) for an additional 75 min leakage period. The greatest amount of leakage occurred after transfer from 0·9 M fructose to water, and lesser amounts leaked from the slices transferred from fructose to sorbitol and then to water. Apparently, during the sorbitol incubation some of the sucrose (12–14 μmoles) was transported into storage. During incubation in 0·9 M fructose, sucrose accumulated in the cytoplasmic synthesis compartments of parenchyma cells because both leakage (transport to the vascular system) and storage (transport into vacuoles) was inhibited. When the slices were transferred to 0·3 M or 0·6 M sorbitol this inhibition was reduced and both leakage and storage took place; thus, there was a smaller amount of sucrose available for leakage when the slices were subsequently transferred to water.

Clearly, the results of Figs. 3 and 4 show that sucrose leakage occurred in a mass flow of solution caused by the influx of water into the cells when they were transferred to water or to a more dilute solution.

Some Properties of the Mass Flow Leakage System

We interpret the results presented so far to mean that a mass flow of solution can occur from the mesophyll cells (since we presume this is where sucrose is accumulated) through the vascular system and into the bathing solution. Such a flow of solution would be expected to carry substances other than sucrose. Substances found in the bathing solution at the end of the leakage period are shown in Table 3. Following fructose incubation, substances leaking in the greatest amounts were (in order of abundance) sucrose, nitrogenous compounds, K^+, inorganic phosphate and Mg^{2+}. Fructose (*ca.* 180 μmoles), of course, also was present in the bathing solution. Since the slices were rinsed only once with 10 ml of water during the transfer from 1·0 M fructose to water, some of this fructose came from the extracellular space but much of it must have leaked either through the vascular system with the mass flow of solution or through the plasma membranes (see Discussion and Ref. 6). Only nitrogenous compounds leaked to any significant extent when the slices were incubated in water instead of fructose (Table 3).

A flow of solution through the scutellum cells is probably a normal event in the intact seedling. However, the flow rate generated upon transferring the slices from 0·9 M fructose to water was undoubtedly much higher than normal and might well have resulted in injury to the cells. To test whether extensive injury occurred the slices were subjected to three

TABLE 3. COMPOSITION OF THE BATHING SOLUTION AT THE END OF THE LEAKAGE PERIOD*

Substance	Amount (μmoles) appearing in H_2O bathing solution following incubation in:	
	H_2O	1·0 M Fructose
Sucrose	<0·2	41·8 ± 2·4
Glucose	<0 2	1 9 ± 0 2
Inorganic P	<0 3	6 2 ± 0·3
Organic P	<0 2	2 1 ± 0·5
Total N (as NH_3)	7 1 ± 2·1	16·9 ± 1·4
K^+	0·4	7·5 ± 0·9
Na^+	1·2	1·5 ± 0 3
Mg^{2+}	0·4	4 0 ± 0 8
Ca^{2+}	<0 2	0 5 ± 0 2
Mn^{2+}	<0 1	<0·1

* The slices (1·0 g fresh wt.) were incubated at 30° in water or 1·0 M fructose for 3 hr. At the end of this period the bathing solution was removed, the slices were washed with 10 ml of water and then 10 ml of water was added to the flasks The slices were incubated at 30° for an additional 90 min and then the bathing solutions were collected for analyses. Seven groups of slices were used to obtain the data for the first five substances listed. The cation data for the fructose flasks were obtained from four groups of slices whereas, for the water flasks, the data were obtained from a solution prepared by pooling equal portions of seven bathing solutions.

[6] L. A. GARRARD and T E. HUMPHREYS, *Nature, Lond.* **207**, 1095 (1965).

1·0 M fructose–water cycles. During each cycle sucrose production and sucrose leakage were determined (Table 4). Sucrose production remained at about the same level during the first two fructose incubation periods but declined by about 25 per cent during the third period. Sucrose leakage into water declined about 20 per cent after both the second and the third fructose incubations. Judging from the sucrose production data, mass flow of solution did not cause extensive damage to the cells. Sucrose production also declines after long periods of incubation of scutellum slices in 0·2 M or 0·5 M fructose;[3,7] however, it is not clear in these cases whether production declined as a result of cell damage or because the rate of sucrose synthesis is controlled by the sucrose level in the tissue. In experiment C of Table 4, the tissue sucrose content increased 33 per cent during the 9 hr 45 min experimental period.

TABLE 4. SUCROSE PRODUCTION AND LEAKAGE DURING REPEATED 1·0 M FRUCTOSE–WATER CYCLES*

Incubation sequence	Sucrose, μmoles	
	produced	leaked
A. First 2-hr period in 1·0 M fructose, followed by 75 min in H_2O	55·2	
	—	42·5
B. Second 2-hr period in 1·0 M fructose, followed by 75 min in H_2O	56·5	
	—	33·9
C. Third 2-hr period in 1·0 M fructose, followed by 75 min in H_2O	42·9	
	—	26·0

* Five groups of slices (each, 1·0 g fresh wt.) were used. One group was killed and extracted at the beginning to obtain the initial tissue sucrose level and one group was killed at the end of both the A and the B cycles. The remaining two groups were used to obtain the leakage data (the leakage data in the table is an average from these two groups) and were killed at the end of the C cycle to obtain the final tissue sucrose level. Both incubations (fructose and water) were at 30°. The procedures were the same as those described in the footnote, Fig. 1, except that three cycles instead of one were run.

In a previous paper[2] it was shown that sucrose leakage into Tris buffer plus 0·1 M fructose was greatly depressed if the slices were given a prior period of incubation in water or 0·1 M fructose. We interpreted these findings as being the result of the formation of phloem plugs during the prior incubation, and we conjectured that phloem plug formation was inhibited by Tris.[2] Thus, when slices are incubated in 0·1 M fructose the newly synthesized sucrose can be transported, as it is formed, either out of the tissue via the phloem (when Tris is present to prevent phloem plug formation) or, if the phloem is plugged, into the storage vacuoles. In contrast, during incubation of slices in concentrated fructose solutions sucrose accumulates and the accumulated sugar leaks when the slices are placed in water or in a

[7] T. E. HUMPHREYS and L. A. GARRARD, *Phytochem.* 7, 701 (1968).

more dilute solution (e.g. Figs. 3 and 4). We suggest that the conduit for this leakage is the same as that utilized when the slices are placed in Tris plus 0·1 M fructose; namely, the phloem.[2]

If our interpretation is correct, then subjecting the slices to a water incubation before placing them in concentrated fructose solutions should introduce phloem plugs and decrease the subsequent leakage. The results of such an experiment are shown in Fig. 5. In this experiment the slices were incubated in fructose (0·5 M or 1·0 M) with or without a prior

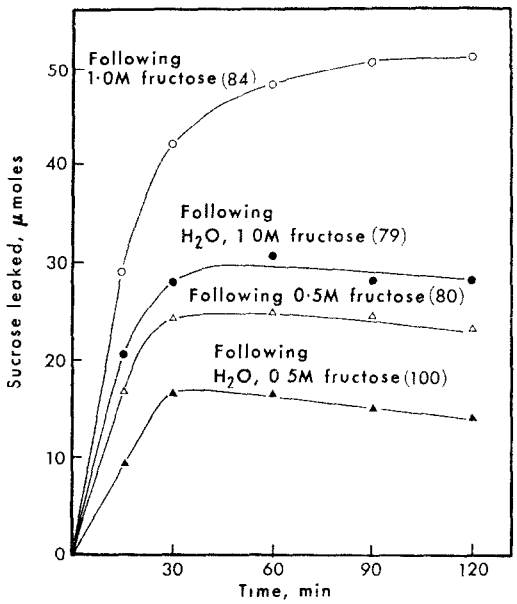

FIG. 5. THE EFFECT OF A PRIOR WATER INCUBATION ON THE ABILITY OF THE SLICES TO PRODUCE AND LEAK SUCROSE.

The slices (1·0 g fresh wt.) were placed in fructose (1·0 M or 0·5 M) at 30° either immediately after their preparation or after a 3-hr incubation in water at 30°. After 3 hr in fructose, the slices were transferred to water (time zero on graph) and sucrose leakage measured. The figures in brackets show the amount of sucrose produced during the fructose incubation.

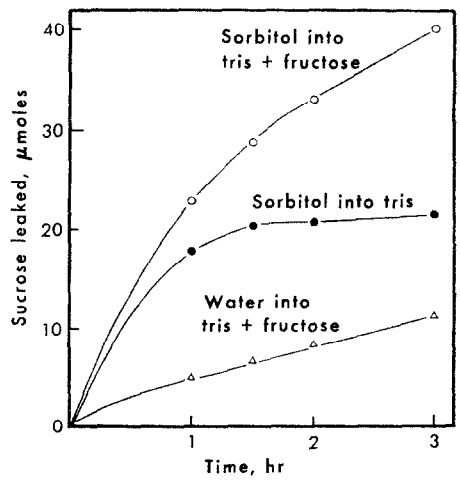

FIG 6. THE PROTECTIVE EFFECT OF SORBITOL ON THE LEAKAGE PROCESS.

The slices (1·0 g fresh wt.) were incubated at 30° for 1 hr in water or in 1 0 M sorbitol. At the end of this period, the slices were transferred (time zero on the graph) to Tris buffer (0·06 M, pH 7·5) plus fructose (0 1 M) or to Tris buffer alone.

3-hr incubation in water. After 3 hr in fructose the slices were put in water and sucrose leakage was measured. Sucrose leakage, but not sucrose production, was decreased (30–40 per cent) by incubating the slices in water prior to the fructose incubation. It appears from these results that the leakage channels become partially plugged during incubation in water. Note, however, that the leakage channels remained open after incubation in 1·0 M fructose or sorbitol (Fig. 1) and during three fructose–water incubation cycles over a 9 hr 45 min period (Table 4). Apparently, concentrated sugar or sugar alcohol solutions prevent the formation of plugs or perhaps the increased osmotic potential of the cytoplasm following incubation in concentrated solutions supports a turgor pressure sufficient to break through the plugs. The results shown in Fig. 6 illustrate the protective action of sugar alcohols

on the sucrose leakage process. In this experiment the slices were incubated for 1 hr in water or in 1·0 M sorbitol before being put in Tris or Tris plus 0·1 M fructose. Sucrose leaked at a low rate into Tris–fructose following incubation in water whereas a high rate of leakage was obtained following incubation in sorbitol. The leakage following sorbitol incubation was initially about as rapid as sucrose production, but after 1 hr the rate declined to only about one-half the rate of sucrose production. Similar curves of sucrose leakage have been obtained from slices placed in Tris–fructose immediately after their preparation,[2] so there was little or no damage to the leakage channels during the sorbitol incubation. The sucrose leakage curve (middle curve, Fig. 6) obtained from slices transferred from sorbitol to Tris is similar to curves obtained with fresh slices placed in HCl, Tris or phosphate.[1, 2] We have suggested that before leakage occurred this sucrose was in the vascular tissue, a place from which it could be easily transported in contrast to the bulk of the tissue sucrose which was sequestered in the vacuoles.

DISCUSSION

The sucrose leakage described in this paper is thought to be the result of mass transport from the mesophyll cells through the vascular tissue and into the bathing solution. The fact that little sucrose leaked from the whole scutellum until the root–shoot axis was removed (Tables 1 and 2) is convincing evidence that vascular tissue is involved in the leakage, and the lability of the process (Figs. 5 and 6, Ref. 2) is suggestive of the phloem as the vascular conduit. The proportionality between the amount of sucrose leakage and the concentration drop of the bathing solution in switching from the fructose solution to a sugar alcohol solution or to water (Figs. 3 and 4) clearly shows that an influx of water into the cells is necessary for leakage. Mass flow of solution, therefore, was the mechanism of sucrose transport in these experiments. The flow must have been caused by the sudden increase in turgor pressure which occurred upon transfer of the scutellum (whole or sliced) to a more dilute solution.

The following pressure-flow transport scheme is consistent with the results of this and previous papers.[1,2,6] When the scutellum slices are placed in a fructose solution, fructose enters the hexose space of the cell[6] until the fructose potentials of the hexose space and the bathing solution are equal. Exogenous hexoses and sugar alcohols readily enter the hexose space presumably by free diffusion through pores in the plasmalemma which are, however, too small to allow passage of sucrose.[6–9] For the purposes of this scheme, we consider the hexose space to include all the cytoplasm but not the vacuoles. The vacuoles shrink, therefore, until the water potentials in the bathing solution, cytoplasm and vacuoles become equal.[10] Sucrose production, initiated by the presence of fructose, continuously changes the concentrations of the cytoplasmic and vacuolar solutions and causes an increase in turgor; there is a concomitant adjustment in the relative volumes of the cellular compartments depending on the distribution of the newly synthesized sucrose. In high concentrations of fructose (e.g. 1·0 M) most of the newly synthesized sucrose remains in the hexose space.[3] When these slices are transferred to a more dilute solution water enters the cells and the turgor pressure increases. The turgor pressure causes a flow of solution (containing fructose, the compounds and inorganic ions listed in Table 3, and most certainly other substances)

[8] T. E. Humphreys and L. A. Garrard, *Phytochem.* **8**, 1055 (1969).
[9] T. E. Humphreys and L. A. Garrard, *Phytochem.* **9**, 1715 (1970).
[10] P. J. Kramer, E. B. Knipling and L. N. Miller, *Science* **153**, 889 (1966).

through the mesophyll cells (via the plasmodesmata), into the sieve tubes (via the plasmodesmata), through the sieve tubes (via the pores in the sieve plates) and into the bathing solution. As the solutes leave the cell a positive, though decreasing, turgor is maintained by vacuolar expansion. Upon transferring the slices from fructose to water (or to a more dilute solution) there is a turgor increase and a resultant pressure-flow of solution because water moves across the plasmalemma more rapidly than fructose can exit through the pores in the plasmalemma; therefore, fructose moves out of the cell with the mass flow of solution.

An analysis of the experiment of Fig. 3 is given in Table 5. This analysis is based on the assumption that the above scheme is correct, additional assumptions and approximations are given in the footnotes to Table 5. The analysis satisfactorily accounts for the quantitative relationships between the amount of sucrose leaked and the magnitude of the concentration difference between the initial fructose solution and the final, more dilute, mannitol solution.

TABLE 5. ANALYSIS OF THE EXPERIMENT OF FIG. 3

	Treatment sequence	Fraction of cell vol.* cytoplasm	vacuole	Osmotic potential	Fraction of cytoplasmic sucrose† Left	Leaked	Amount of sucrose leaked‡
A.	Fructose (0·9 M)	0 9	0·1	x	1·0	—	—
	Mannitol (0·3 M)	0·7	0·3	$1/3\ x$	$1/3 \times 7/9 = 0{\cdot}26$	0·74	$0\ 74\ y$
B.	Fructose (0.9 M)	0·9	0 1	x	1·0	—	—
	Mannitol (0 6 M)	0·85	0·15	$2/3\ x$	$2/3 \times 85/90 = 0{\cdot}63$	0·37	$0{\cdot}37\ y$
C.	Fructose (0·6 M)	0·85	0·15	$2/3\ x$	1·0	—	—
	Mannitol (0·3 M)	0·7	0·3	$1/3\ x$	$1/2 \times 70/85 = 0{\cdot}41$	0 59	0 59 $(6/9\ y)$

* It was assumed that the fructose (or mannitol) concentrations of the bathing solution and cytoplasm were the same, but that these substances did not penetrate into the vacuole The vacuole was considered to contain only sucrose which was at the same concentration as the fructose or mannitol of the bathing solution. The slices initially contained about 90 μmoles of sucrose/ml of tissue water and this figure was used to calculate the fraction of the cell volume occupied by the vacuole.

† It was assumed that the decrease in osmotic potential of the vacuole was a result of an increase in vacuolar volume whereas in the cytoplasm the osmotic potential decreased as a result of the mass flow of solution out of the cell. We have disregarded matric potential in these calculations.[10] The mass flow of solution removed amounts of solutes in proportion to their concentration so that the osmotic potential of the cytoplasmic sucrose decreased to the same extent as the total osmotic potential. Therefore, the fraction of the original cytoplasmic sucrose (that present in the cytoplasm at the end of the fructose incubation) left in the cell at the completion of the mass flow is equal to the fractional osmotic potential multiplied by the fractional cytoplasmic volume.

‡ The amount of cytoplasmic sucrose at the end of the 3-hr incubation in 0·9 M fructose is equal to y. Based on the data of Figs. 3–5 (also, see Ref. 3) we estimate that after 3 hr in 0·6 M fructose the amount of cytoplasmic sucrose was only $6/9\ y$. Thus, by this analysis, treatments B and C would result in about equal amounts of sucrose leakage as is required by the results shown in Fig. 3.

However, when slices are transferred from fructose to water, estimates of the final cytoplasmic volume and osmotic potential are difficult to make; nevertheless, assuming the vacuole swells from 0·3 to 0·8 of the cell–water volume when the slices are transferred from 0·3 M fructose to water, about 90 per cent of the cytoplasmic sucrose would be removed from the cell with the mass flow (i.e. $3/8 \times 2/7$ of the sucrose would be left in the cytoplasm). More than 90 per cent of the cytoplasmic sucrose would leak from slices transferred to water from fructose solutions of greater than 0·3 M concentration.

We have assumed in this and previous papers[3, 4] that all of the cytoplasmic sucrose leaked into water and, on this assumption, we have calculated the amount of sucrose transported into storage (vacuoles) and left in the hexose space (cytoplasm) during a period of sucrose synthesis in which the slices were incubated in fructose solutions. Actually, the amount of sucrose leaked into water is probably an underestimate of the amount that was present in the cytoplasm for two reasons: first, as stated above, 10 per cent or less of the sucrose would remain in the cytoplasm after the pressure-flow was completed and second, the loss of fructose through pores in the plasmalemma would decrease the amount of solution flow and leave more sucrose in the cytoplasm. It is likely that a turgor pressure greater than a certain threshold pressure is necessary for solution flow and that the fructose of the cytoplasm is the main solute supporting the turgor pressure. Exit of fructose either with the mass flow of solution or through the pores in the plasmalemma would cause the turgor to fall below the threshold value. The amount of fructose lost through the plasmalemma would be proportional to the fructose concentration; therefore, although this loss would decrease the actual amounts of sucrose leaked, the relative amounts of leakage would remain the same.

A considerable volume of solution must flow through the tissue when the slices are transferred to a more dilute solution. An estimate of this volume can be obtained by calculating the flow in two extreme cases and taking an average; assume that during the entire flow the solution leaves the cell at its initial concentration (case 1) or at its final, more dilute, concentration (case 2). Consider a cell or tissue of unit volume and using the experiment in which the slices were transferred from 0·9 M fructose to 0·3 M mannitol (Fig. 4, Table 5) as an example, the following calculations give the volume of flow.

Case 1. 0·9 initial cytoplasmic vol. — 0·7/3 vol., at initial concentration, left in cell = 0·67 vol. of flow.

Case 2. 0·9 Initial cytoplasmic vol. × 3 dilution factor — 0·7 final cytoplasmic vol. = 2·0 vol. of flow.

The average volume of flow would be, therefore, 1·34 times the cell volume (the volume contained within the plasma membranes). Estimating that the cell volume of 1 g of slices is 0·8 ml, the volume of solution flowing through the cytoplasm during the entire flow period (Fig. 4) would be 1·07 ml or nearly twice the final volume of the cytoplasm (0·56 ml). Transfer of slices from 1·0 M fructose to water would result in a greater volume of flow. In these calculations the assumption is made, of course, that fructose is not lost from the cell by diffusion through the plasma membranes.

The above analysis requires that there are distinct pools of sucrose within the scutellum. We suggest[1, 2] that there are three such pools: a storage pool located in the vacuoles of the mesophyll cells, a cytoplasmic pool in the mesophyll and a pool located in the cells of the vascular tissue which may be both cytoplasmic and vacuolar. The vascular pool is emptied into the bathing solution if fresh slices are placed in Tris, HCl or inorganic phosphate solutions (Refs. 1, 2, 8 and middle curve, Fig. 6), and leakage curves similar to these are obtained when the bathing solution contains high concentrations of fructose (0·8 M–1·0 M) in addition to Tris or HCl.[1, 8] In the latter case, however, there is also an accumulation of sucrose which leaks when the slices are transferred to water, so we visualize accumulation sites in the mesophyll cytoplasm and conjecture that the concentrated solutions disrupt the movement of materials between the mesophyll tissue and the vascular tissue. The disruption probably takes the form of increased resistance to flow since increasing the turgor by trans-

ferring slices to a more dilute solution (Figs. 3 and 4) results in a flow of solution. Another reason for placing the accumulation site of cytoplasmic sucrose in the mesophyll is that relatively large amounts of sucrose are accumulated and the great majority of the cells in the scutellum are mesophyll parenchyma cells.

There is a third pool of sucrose. This pool does not readily leak from the scutellum; we call this the storage pool and believe it to occupy the vacuoles although we have no evidence that it does indeed reside there. However, this pool certainly is separate from the mesophyll cytoplasmic pool for when slices (fresh or after a 3-hr incubation in 0·1 M fructose) were put in 1·0 M sorbitol for 1 hr and then put in water only a small amount of sucrose (about 12 μmoles in each case) leaked into the bathing solution (unpublished results). This experiment is similar to that of Fig. 1 (also, see Ref. 3) except for the use of 0·1 M instead of 1·0 M fructose. We conclude that in fresh slices most of the sucrose is in storage, that the sucrose synthesized during incubation of the slices in 0·1 M fructose is transported into storage and that the pressure-flow developed upon transferring the slices from sorbitol to water will not move stored sucrose.

When fresh slices are placed in 0·1 M fructose containing Tris or HCl sucrose leakage occurs at a rate that may reach the rate of sucrose production.[1, 2] Tris and HCl appear to keep the sieve tubes open (presumably by removing divalent cations[2]) which would lower the threshold pressure necessary for mass flow to occur. However, in contrast to the results of this paper concerning leakage following incubation in concentrated fructose solutions, we have no evidence that this Tris or HCl induced leakage is a result of pressure-flow. Indeed, transport of sucrose through the mesophyll may consist of diffusion across plasmodesmata followed by rapid mixing within each cell as a result of cyclosis;[11] phloem companion cells and phloem parenchyma cells may actively accumulate sucrose from the mesophyll and in turn load the sieve tubes.[12–14] In this scheme pressure-flow would be limited to the sieve tubes.

The results of this paper indicate that a pressure-flow occurred when the slices were transferred to a more dilute bathing solution. However, this was an artificial situation in which rather high turgor pressures were suddenly applied; therefore, the results of this paper do not constitute evidence that pressure-flow is the usual or a normal mechanism of sucrose transport. On the other hand, the results of this paper are evidence that a pressure-flow may not be limited to the sieve tubes but can occur among mesophyll cells and between mesophyll cells and vascular cells. Indeed, if our interpretation of these results is correct it appears from the sucrose production data of Table 4 that a sudden surge of solution through the mesophyll cells caused little damage. A pressure-flow transport system containing a conduit made up not only of sieve tubes but also of other cells such as companion cells and phloem parenchyma would have several advantages over a system in which the pressure-flow was restricted to only the sieve tubes: among these being the increase in surface at the phloem loading and unloading locations, the pressure regulating effects of the vacuoles and the secondary control of the availability of solutes for transport exerted at the tonoplast.

Esau, Cronshaw and Hoefert[15] found yellows virus particles in the parenchyma cells and in mature sieve elements of the minor veins of sugar beet leaves; moreover, virus par-

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ticles were found in the pores of the sieve plates and in plasmodesmata both between parenchyma cells and between sieve elements and parenchyma cells. These authors suggest that virus particles move with the flow of solution from cell to cell through sieve pores and through plasmodesmata. The plasmodesma is a complex structure which appears to contain a central core restricting the lumen.[15,16] Robards[16] concludes from studies on willow that there is no continuity through the plasmodesmata between the cytoplasm of adjacent cells. However, the fact that virus particles have been shown to be in adjacent cells and in a plasmodesma connecting these two cells does not support this conclusion.[15] Clearly, the interchange of materials between cells either by way of the plasmodesmata or by way of the plasmalemma–cell wall–plasmalemma is poorly understood.[11,17]

Peel and Weatherley[18] analyzed the exudate from an aphid stylet inserted in a single sieve tube of willow and found sucrose, nitrogenous compounds and K^+ to be the three most abundant constituents. Results similar to this were found for scutellum leakage (Table 3). In fact, the molar ratio of sucrose to K^+ was about 6 in both the willow exudate[18] and the scutellum exudate (Table 3); however, the amount of organic nitrogen relative to sucrose was much higher in the willow exudate. In willow the sieve tube sap contained carboxylic acids but no phosphate. However, organic and inorganic phosphates do occur in phloem exudates,[17] and Bieleski[19] concludes that inorganic phosphate is the primary form in which phosphorus moves in the phloem.

EXPERIMENTAL

Plant material. Maize grains (*Zea mays* cv. Funks G-76) were soaked in running tap water for 24 hr and then placed on moist filter paper in the dark at 24–25° for 72 hr. The scutella were excised from the germinating grains and cut transversely into slices 0·5 mm or less in thickness. The slices were washed in distilled water until the wash water remained clear, and then were blotted on filter paper and weighed in groups of 1 g. The preparation of the whole scutellum is given in the footnote of Table 1.

Experimental procedure. Detailed procedures for these experiments are given in the Tables and Figures. Each group of slices (1·0 g fresh wt.) was placed in 10·0 ml of the appropriate bathing solution and incubated at 30° in a 'Gyrotory' water bath (New Brunswick Scientific Company, New Brunswick, N.J.). Procedures for the determination of sucrose leakage and for the preparation of the ethanolic extracts of the tissue slices have been described previously.[20]

Analytical. The sucrose contents of the bathing solutions and tissue extracts were determined by analyzing these solutions for glucose before and after invertase treatment. Glucose was determined by the glucose oxidase method. Inorganic phosphorus was determined by the method of Fiske and SubbaRow;[21] total phosphorus was determined after digesting the sample in H_2SO_4 at 160° for 3 hr. Total nitrogen was determined by Nesslerization following sample digestion in H_2SO_4. Cation concentrations were determined by atomic absorption spectroscopy. The cation analyses were performed by Dr. H. L. Breland of the Soils Department, University of Florida.

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