Phytochemistry, 1971, Vol. 10, pp. 981 to 995. Pergamon Press. Printed in England.

SUCROSE LEAKAGE FROM THE MAIZE SCUTELLUM: EVIDENCE FOR THE PARTICIPATION OF THE PHLOEM*

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(*Received* 27 *July* 1970)

Abstract—Sucrose leaked from maize scutellum slices when they were incubated in fructose. The rate of leakage declined with time, but the initial rate could be maintained in the presence of HCl, ATP or certain buffers. The leakage process was a labile one and the ability of cells to leak (but not produce) sucrose declined during incubation of the slices in water at 30°. The leakage process was protected, however, during incubation in ice water. Sucrose leakage from whole scutella had properties similar to leakage from slices but had only about one-third the rate. The whole scutellum leaked appreciable sucrose only after the root–shoot axis was removed. The compounds which increased leakage also have the ability to displace or to form complexes with cations, and it appears that their effect on leakage is due to the removal of Mg^{2+} (and possibly Ca^{2+}) from the slices. Evidence is presented that there is a pool of leakable sucrose which, when the leakage process is functioning, can be emptied into the bathing solution. The pool level is maintained, however, when fructose is present as a source of sucrose. The results presented are consistent with the idea that sucrose leakage originates in the sieve tubes of the phloem and is the end result of a series of events which includes intercellular sucrose transport, vein loading and phloem transport.

INTRODUCTION

IN A PREVIOUS paper[1] we suggested that sucrose leakage from maize scutellum slices bathed in HCl and fructose came from vascular tissue rather than through the plasma membranes of the mesophyll, parenchyma cells which make up the bulk of the scutellum. This suggestion implies that sugar transport processes in the slices are functioning in a manner similar to those of the intact seedling except that the bathing solution instead of the root–shoot axis serves as the ultimate sucrose sink. If these ideas are correct, maize scutellum slices should prove to be excellent material for studies of vein loading and phloem transport.

In this paper we present the results of our studies on the characteristics of the sucrose leakage process. These results constitute further evidence for a role of vascular tissue in sucrose leakage from sliced or whole scutella.

RESULTS

Sustained Sucrose Leakage in the Presence of Fructose and Tris or Phosphate Buffers

Previous studies[1,2] showed that both HCl (0·01 M) and Tris buffer (0·06 M, pH 7·5) caused an increased leakage of sucrose from scutellum slices. This occurred whether or not fructose was present in the bathing solution, but the rate of leakage was sustained in the presence of fructose. The effects of three buffers (pH 7·5) on sucrose leakage in the absence

* Florida Agricultural Experiment Station Journal Series No. 3637. This investigation was supported, in part, by a research grant (No. AM 07299-06) from The National Institute of Arthritis and Metabolic Diseases, U.S. Public Health Service.

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[1] L. A. GARRARD and T. E. HUMPHREYS, *Phytochem.* **9**, 1715 (1970).

[2] T. E. HUMPHREYS and L. A. GARRARD, *Phytochem.* **8**, 1055 (1969).

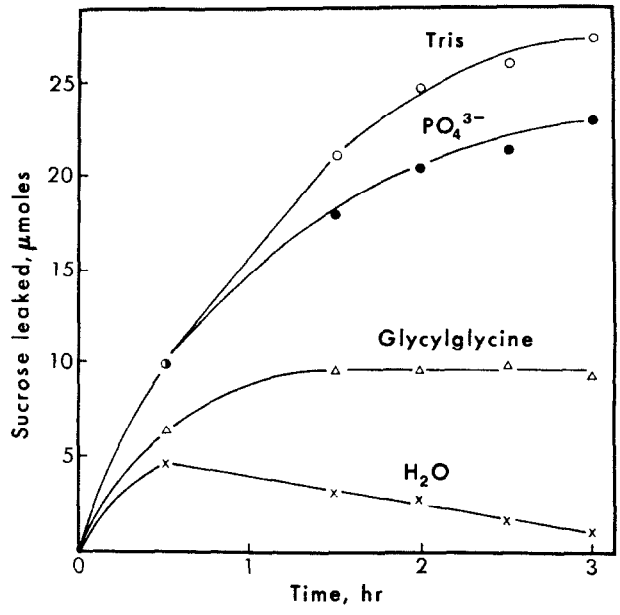

FIG. 1. THE EFFECT OF BUFFERS AT pH 7·5 ON THE LEAKAGE OF SUCROSE FROM SCUTELLUM SLICES. THE SLICES (1·0 g fr. wt.) WERE INCUBATED IN WATER OR BUFFER (0·06 M, pH 7·5) AT 30°.

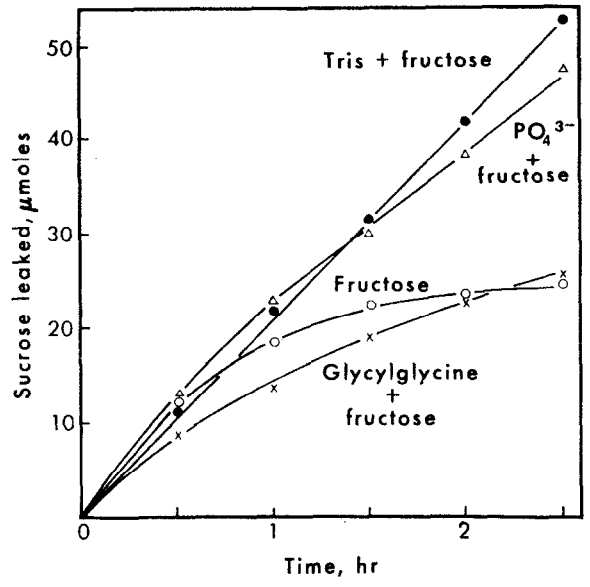

FIG. 2. THE EFFECT OF BUFFERS AND FRUCTOSE ON THE LEAKAGE OF SUCROSE FROM SCUTELLUM SLICES. THE SLICES (1·0 g fr. wt.) WERE INCUBATED IN FRUCTOSE (0·1M) OR IN FRUCTOSE (0·1 M) PLUS BUFFER (0·06 M, pH 7·5) AT 30°.

or presence of fructose are shown in Figs. 1 and 2. Tris and phosphate buffers caused a marked increase in leakage. Glycylglycine caused only a small increase in sucrose leakage in the absence of fructose (Fig. 1) and had little effect in its presence (Fig. 2). Since glycylglycine strongly inhibits the uptake of sucrose by scutellum slices (unpublished results), the increased leakage in the presence of glycylglycine was probably the result of an inhibition of sucrose reabsorption. Tris and phosphate buffers at pH 7·5 also inhibit sucrose uptake[2,3] but with these buffers there was clearly an increased sucrose leakage. When only fructose

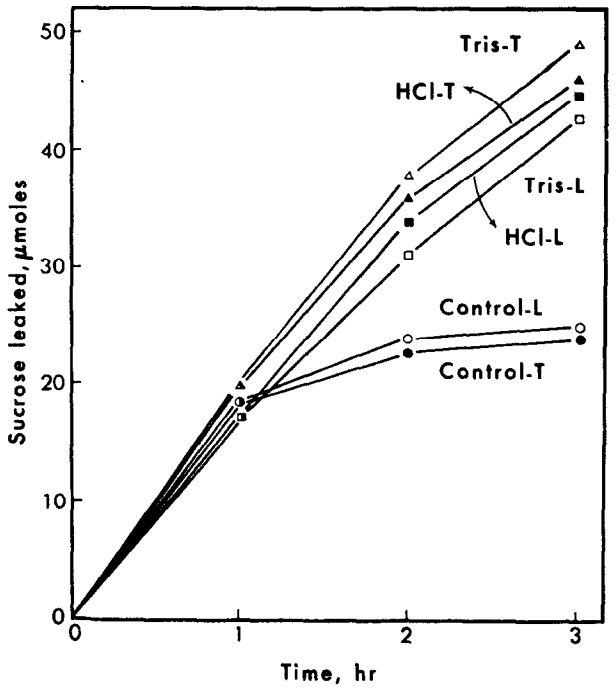

FIG. 3. SUCROSE LEAKAGE FROM LONGITUDINAL SLICES AND FROM TRANSVERSE SLICES. TRANSVERSE (T) SLICES WERE THE KIND USED IN THE OTHER EXPERIMENTS REPORTED IN THIS PAPER. LONGITUDINAL (L) SLICES WERE CUT PARALLEL TO THE LONG AXIS OF THE SCUTELLUM. BOTH KINDS OF SLICES WERE 0·5 mm OR LESS IN THICKNESS. THE SLICES (1·0 g fr. wt.) WERE PLACED IN TRIS (0·06 M, pH 7·5) PLUS FRUCTOSE (0·1 M), IN HCl (0·01 M) + FRUCTOSE (0·1 M) OR IN THE CONTROL FLASKS WHICH CONTAINED ONLY FRUCTOSE. THE SLICES WERE INCUBATED AT 30°.

was present in the bathing solution the initial rate of sucrose leakage was as high as when Tris or phosphate buffers were also present (Fig. 2). The buffers, therefore, did not initiate the leakage but were necessary to sustain it.

In the experiment of Fig. 2 the amount of glucose found in the bathing solution was, in all cases, less than 2 μmoles. When the slices were bathed in HCl and fructose (e.g. see Fig. 3) 7–8 μmoles of glucose were found in the bathing solution at the end of the 3-hr incubation period. This glucose probably came from breakdown of sucrose in the acid bathing solution. Paper chromatography of the bathing solutions revealed only three carbohydrate spots: glucose, fructose and sucrose.

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

Evidence that Tris affects Vascular Tissue

The effects of Tris on sucrose leakage from scutellum slices, from whole scutella and from whole scutella with the root–shoot axes attached are shown in Table 1. In the presence of fructose, Tris caused an increased sucrose leakage from whole scutella only when the

TABLE 1. EFFECT OF TRIS ON SUCROSE LEAKAGE FROM THE MAIZE SCUTELLUM

Condition of scutellum*	Sucrose (μmoles) leaked into:†		
	Fructose	Tris	Tris† fructose
Sliced	28·2	27·3	58·8
Whole (excised)	3·1	—	22·0
Whole (attached to root–shoot axis)	3·6	5·5	4·2

* Plant material was prepared as described in the Experimental section. Ten excised scutella were used per flask. At the end of the experiment each group of 10 scutella was weighed (*ca.* 0·9 g) and the results expressed on a per g fr. wt. basis. The numbers of whole scutella with attached root–shoot axes used were: eight for the fructose, three for the Tris and 11 for the fructose + Tris treatments. At the end of the experiment the scutella were removed and weighed. The results are expressed on a per g fr. wt. basis.

† The concentrations of the bathing solutions were: fructose (0·1 M) and Tris (0·06 M, pH 7·5). The tissue was incubated for 3 hr at 30° (sliced and whole scutellum) or 24° (whole scutellum with the root–shoot attached).

root–shoot axes were removed. This leakage was only about one-third of that obtained from the slices. When the root–shoot axis was attached, the scutellum leaked only small amounts of sucrose and there was little difference in the amounts leaked into fructose, Tris or Tris plus fructose. Clearly, this indicates that in the presence of Tris plus fructose the sucrose was leaking through the single cut surface formed upon removal of the seedling axis. This cut severed the main vascular bundle connecting the vascular system of the scutellum with that of the seedling axis. We suggest that the severed bundle was the source of the sucrose leakage. The burden of this paper is to present evidence that there is a sucrose leakage process involving the vascular system as contrasted to faulty plasma membranes.

We suggest, as we did previously,[1] that in the slices the source of the sucrose leakage is also the severed vascular bundles, and that the higher rate of leakage obtained from the slices is due to the larger number of severed vascular bundles and to the much shorter pathway between sucrose producing mesophyll cells and the open end of a severed bundle. Although the main vascular bundle runs parallel to the long axis of the scutellum, the vascular system is highly ramified and branches and branchlets arise from the main bundle throughout its length.[4,5] These branches and branchlets extend to within two or three cell rows beneath the epithelial layer.[4] The slices used in these and previous studies were prepared by making transverse (at right angles to the long axis of the scutellum) cuts and would contain, therefore, short segments of the main vascular bundle open to the bathing solution at both ends. Leakage may have occurred through these severed main bundles but since the

[4] E. SARGENT and A. ROBERTSON, *Ann. Botany, Lond.* **19**, 115 (1905).
[5] G. S. AVERY, JR., *Botan. Gaz.* **89**, 1 (1930).

vascular system is highly ramified and since a large proportion of the mesophyll cells are some distance from the main bundle it seems likely that the vascular branches and branchlets were also involved. To test this, both transverse and longitudinal (cut parallel to the long axis of the scutellum and thus parallel to the long axis of the main vascular bundle) slices were prepared and placed in fructose or in fructose plus Tris or HCl. The amounts of sucrose leaked from these two kinds of slices were nearly the same (Fig. 3). Therefore, it appears that if, in fact, sucrose leakage is through severed vascular bundles, the small bundles which ramify throughout the mesophyll tissue are able to function in this regard.

Evidence for a Pool of Leakable Sucrose within the Slices

A sustained, high rate of sucrose leakage as a result of incubating the slices in Tris plus fructose is shown in Fig. 4. Over the 6-hr period of the experiment, the tissue sucrose stayed at about a constant level and, therefore, the amount of sucrose that leaked and the net amount produced were about equal. Note that when the bathing solution contained Tris but no fructose (Fig. 1) an appreciable amount of the sucrose originally present in the slices leaked into the bathing solution whereas when fructose was present with the Tris the original amount of tissue sucrose was maintained (Fig. 4). The results of Fig. 1 can be reconciled with those of Fig. 4 if it is assumed that the slices contain a pool of sucrose which

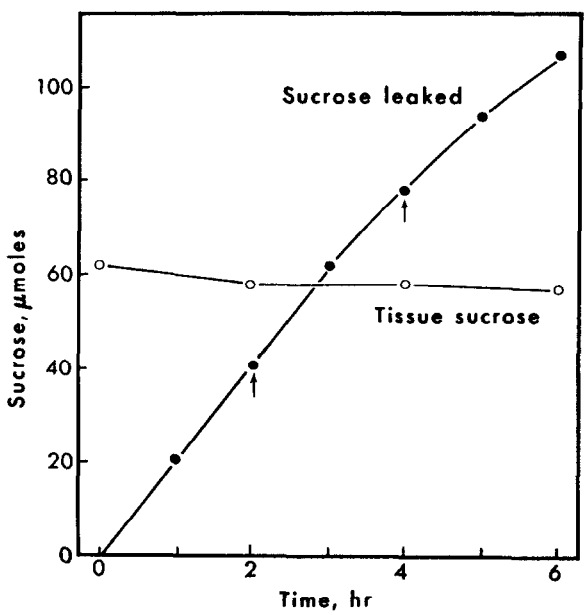

FIG. 4. SUCROSE LEAKAGE AND TISSUE SUCROSE DURING INCUBATION OF SLICES IN FRUCTOSE PLUS TRIS. THE SLICES (1·0 g fr. wt.) WERE INCUBATED IN FRUCTOSE (0·1 M) PLUS TRIS (0·06 M, pH 7·5) AT 30°. FOUR GROUPS OF SLICES WERE USED; ONE GROUP WAS KILLED AT ZERO TIME AND THE OTHER THREE GROUPS WERE PLACED IN THREE FLASKS CONTAINING TRIS–FRUCTOSE SOLUTION. SUCROSE LEAKAGE WAS DETERMINED AT THE TIMES INDICATED. A GROUP OF SLICES WAS REMOVED AND KILLED AT THE END OF 2, 4 AND 6 hr TO DETERMINE TISSUE SUCROSE. THEREFORE, THE FIRST TWO POINTS ON THE LEAKAGE CURVE WERE OBTAINED BY AVERAGING THE RESULTS FROM THREE GROUPS OF SLICES, THE SECOND TWO POINTS WERE OBTAINED FROM TWO GROUPS OF SLICES AND THE LAST TWO POINTS WERE OBTAINED FROM A SINGLE GROUP OF SLICES. AT THE ARROWS THE BATHING SOLUTIONS WERE REPLACED WITH FRESH TRIS–FRUCTOSE SOLUTION.

resides perhaps in the vascular tissue and from which sucrose is transported into the sieve tubes of the phloem. The pool is depleted by leakage when the slices are incubated in certain buffers or HCl, but the pool level is maintained when fructose also is present as a source of sucrose. We make the additional assumptions that newly synthesized sucrose in the cytoplasm is rapidly transported to the leakable pool whereas stored sucrose in the vacuoles is made available for leakage much more slowly. The results shown in Fig. 5

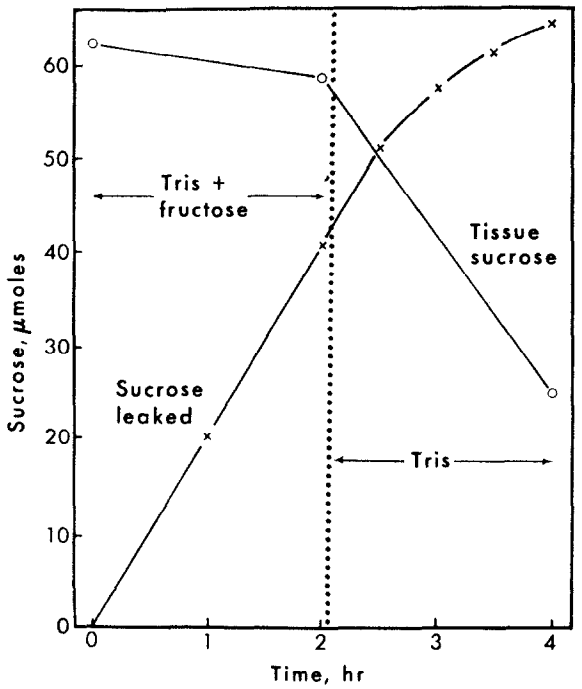

FIG. 5. THE PERSISTENCE OF SUCROSE LEAKAGE AFTER TRANSFER OF THE SLICES FROM TRIS–FRUCTOSE TO TRIS. THE SLICES (1·0 g fr. wt.) WERE INCUBATED AT 30° IN TRIS (0·06 M, pH 7·5) PLUS FRUCTOSE (0·1 M) FOR 2 hr, THEN THE BATHING SOLUTIONS WERE REMOVED, THE SLICES WERE WASHED WITH 10 ml OF TRIS AND 10 ml OF TRIS WERE ADDED. INCUBATION WAS CONTINUED FOR AN ADDITIONAL 2 hr. THREE GROUPS OF SLICES WERE USED: ONE GROUP WAS KILLED AT ZERO TIME, THE SECOND GROUP WAS KILLED AFTER 2 hr AND THE THIRD GROUP AFTER 4 hr.

support these assumptions. In this experiment the slices were initially placed in Tris and fructose. During this period the tissue sucrose level remained approximately constant and sucrose leaked in an amount equal to net sucrose production. When the slices were then transferred to Tris alone, however, the tissue level fell while leakage persisted although the rate declined. It is notable that the leakage curve (Fig. 5) obtained after transferring the slices to Tris is very similar to that shown in Fig. 1. Presumably both curves represent the time course for emptying the leakable sucrose pool.

The Labile Nature of the Leakage Process

The rapid decline in rate of sucrose leakage when the slices were incubated in fructose alone (Fig. 2) and the fact that the rate of leakage sometimes declined after the first hr of

incubation even when Tris or HCl were also present (compare Fig. 3 with Fig. 4) suggest that the leakage process is a labile one. Previously we reported that incubation of the slices in water before the addition of fructose and HCl strongly depressed sucrose leakage.[1] The results of a more detailed experiment on prior incubation are shown in Fig. 6. In this experiment the slices were incubated in water or in fructose for the periods shown and then

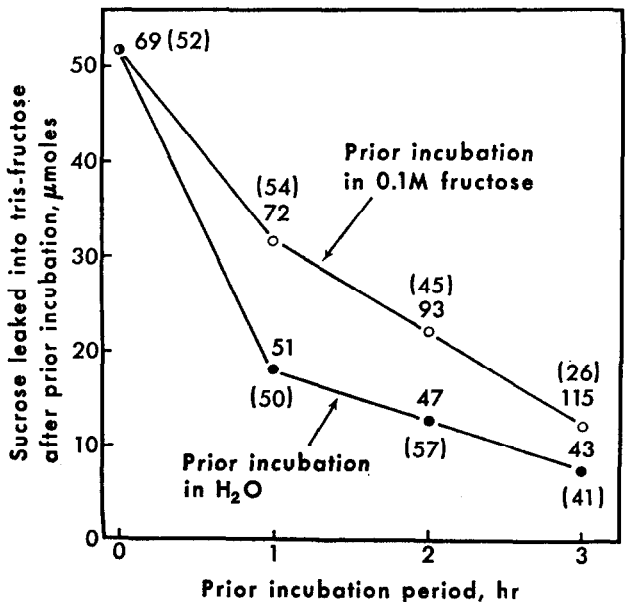

FIG. 6. THE EFFECT OF PRIOR INCUBATION IN FRUCTOSE OR WATER ON THE LEAKAGE OF SUCROSE FROM SLICES INCUBATED IN TRIS–FRUCTOSE. THE SLICES (1·0 g fr. wt.) WERE INCUBATED AT 30° IN WATER OR FRUCTOSE FOR THE LENGTHS OF TIME SHOWN IN THE FIGURE. AT THE END OF THE PRIOR INCUBATION PERIOD THE BATHING SOLUTIONS WERE REMOVED, THE SLICES WERE WASHED WITH 10 ml OF WATER AND THEN 10 ml OF TRIS (0·06 M, pH 7·5) PLUS FRUCTOSE (0·1 M) WERE ADDED. THE SLICES WERE THEN INCUBATED AT 30° FOR 2·5 hr. AT THE END OF THIS PERIOD A SAMPLE OF THE BATHING SOLUTION WAS REMOVED FOR SUCROSE DETERMINATION AND THE SLICES WERE KILLED TO EXTRACT THE TISSUE SUCROSE. A COMPANION SET OF SLICES WAS GIVEN THE PRIOR INCUBATION AND THEN KILLED TO DETERMINE THE TISSUE SUCROSE AT THE START OF THE TRIS–FRUCTOSE INCUBATION. THE NON-BRACKETED FIGURES ARE THE TISSUE SUCROSE LEVELS (μmoles/g fr. wt.) AT THE START OF THE TRIS–FRUCTOSE INCUBATION. THE BRACKETED FIGURES ARE THE NET AMOUNTS OF SUCROSE (μmoles) PRODUCED DURING THE TRIS–FRUCTOSE INCUBATION.

placed in a Tris–fructose solution and the sucrose leakage determined. The tissue levels of sucrose at the time the slices were placed in the Tris–fructose solution and the sucrose production during the Tris–fructose incubation are also shown on the graph. The amount of sucrose leaked decreased with the length of the period of prior incubation in either water or fructose. However, the presence of fructose in the prior incubation medium protected the leakage process slightly. Leakage was not correlated with the tissue sucrose level at the start of the Tris–fructose incubation or with the amount of sucrose produced during the leakage period. Clearly, changes occurred during prior incubation which inhibited leakage and which were not reversed by Tris.

Incubation in water also depressed sucrose leakage from whole scutella when they were

subsequently placed in fructose and Tris or HCl (Fig. 7). In this experiment no more than 5 min elapsed between removing the root–shoot axes and beginning the prior incubation period so that no scutellum was in water more than 35 min before addition of the Tris–fructose or HCl–fructose solutions. However, in a typical experiment using scutellum slices (such as the water prior-incubation experiment of Fig. 6 which required 8 g of slices) the preparation of plant material required the following sequence: whole scutella (minus root–shoot axes) were collected in water (30 min), the scutella were sliced into water (15 min) and

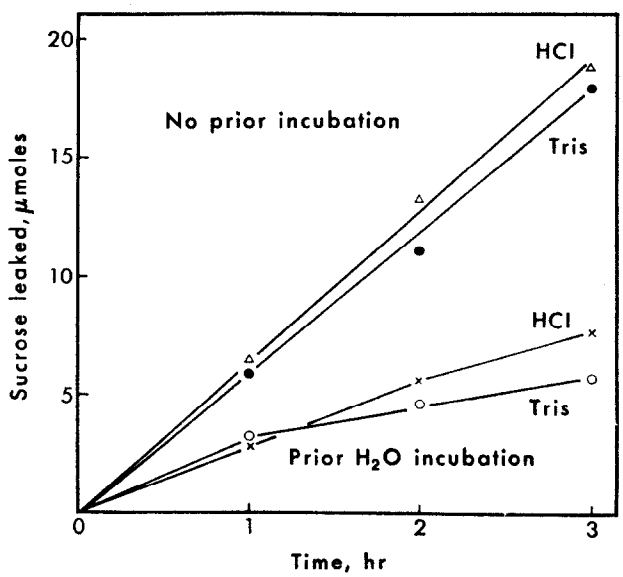

FIG. 7. SUCROSE LEAKAGE FROM WHOLE SCUTELLA. EACH FLASK CONTAINED 10 SCUTELLA. THE SCUTELLA WERE WEIGHED (*ca.* 0·9 g) AT THE END OF THE EXPERIMENT AND THE DATA IN THE FIGURE ARE EXPRESSED ON A g fr. wt. BASIS. THE SCUTELLA WERE INCUBATED AT 30° IN 0·1 M FRUCTOSE SOLUTION WHICH CONTAINED EITHER TRIS (0·06 M, pH 7·5) OR HCl (0·01 M). AS INDICATED ABOVE, SOME SCUTELLA WERE INCUBATED IN WATER (30° FOR 30 min) BEFORE THE ADDITION OF FRUCTOSE PLUS TRIS OR HCl. DURING THIS 30-min INCUBATION IN WATER THE SCUTELLA LEAKED 4·4 μmoles OF SUCROSE. THIS AMOUNT WAS SUBTRACTED FROM THE AMOUNTS OF LEAKAGE OBTAINED FROM THE SCUTELLA RECEIVING NO PRIOR INCUBATION. SCUTELLA INCUBATED IN 0·1 M FRUCTOSE LEAKED ABOUT 4 μmoles OF SUCROSE IN THE FIRST 45 min AND NONE THEREAFTER.

the slices were washed, blotted and weighed (25 min). Therefore, slices in the flask which received no prior incubation (Fig. 6) actually were in water as whole scutella and as slices for a minimum of 40 min and a maximum of 70 min before being placed in Tris–fructose. Since a random sample (1·0 g) of the total group of slices was taken for each flask, an average of 55 min elapsed from the time the root–shoot axes were removed to the time the slices were placed in Tris–fructose. In spite of the long preparation period these slices leaked about as much sucrose as they produced over the 150-min period in Tris–fructose. A simple explanation of these results is provided by the idea that sucrose leakage occurs through cut ends of vascular elements which become plugged during incubation in water but which are kept open in Tris. Whole excised scutella have few cut ends through which the entire vascular system must empty and, therefore, leakage from a whole scutellum is stopped by a few vascular plugs. The slices, on the other hand, have many cut ends serving relatively

short vascular segments. This system is more difficult to plug since in the slices there may be a number of paths bypassing the plugged portion of a vascular element.

The effect of temperature during preparation of the tissue and during the prior incubation period on the subsequent sucrose leakage is shown in Table 2. In these experiments, only slices were used for sucrose leakage determinations. However, where appropriate, both slices and whole scutella were given a prior incubation, after which the whole scutella were sliced before placing the tissue in the Tris–fructose solution. Incubation in ice water was much less deleterious to the leakage process than incubation at 30°. This was true both when slices and when whole scutella were given a prior incubation (Table 2, compare *B* with *C* and *G* with *H* or *D*). The use of ice water during the preparation of the scutella and the slices also increased the leakage (Table 2, *E*, *F*). Note that leakage from these slices was less than that obtained previously (compare *A* or *F*, Table 2 with Fig. 4) and about one-third of the

TABLE 2. EFFECT OF TEMPERATURE DURING PREPARATION AND DURING PRIOR WATER INCUBATION OF SCUTELLA AND SCUTELLUM SLICES ON THE SUBSEQUENT SUCROSE LEAKAGE*

Conditions prior to placing the slices in fructose (0·1 M) plus Tris (0·06 M, pH 7·5)	Sucrose (μmoles)		
	Leakage	Tissue change	Net production
Experiment 1			
A Scutella fresh, slices fresh. Both held at room temp. during preparation.	44·5	23·7	68·2
B Scutella incubated in H_2O at 30° for 1 hr, slices fresh.	24·5	37·5	62·0
C Scutella incubated in H_2O at 1° for 1 hr, slices fresh.	38·3	18·1	56·4
D Scutella prepared at room temp., slices incubated in H_2O at 30° for 1 hr.	12·2	49·1	61·3
Experiment 2			
E Scutella fresh, slices fresh. Both held in ice water during preparation.	46·1	18·0	64·1
F Scutella fresh, slices fresh. Both held at room temp. during preparation.	37·6	23·9	61·5
G Scutella prepared in ice water, slices incubated in H_2O at 1° for 1 hr.	37·6	27·8	65·4
H Scutella prepared at room temp., slices incubated in H_2O at 30° for 1 hr.	9·2	48·6	57·8

* Excising and slicing the scutella and washing, blotting and weighing the slices took about 70 min for eight groups of slices weighing 1·0 g per group. In these experiments the scutella were excised into water at room temp. (about 24°) or into ice water (about 1°), and the slices were placed in (as they were cut) and washed in water at 24° or 1°. In addition, in some cases the scutella or the slices were incubated for 1 hr at 30° or 1°. These conditions are indicated in the Table. Each experiment required eight groups of slices since, for each treatment, two groups of slices were used. At the end of the preconditioning period one group was killed and the other group was placed in fructose plus Tris and incubated at 30° for 3 hr. During this latter period sucrose leakage was measured and at the end of this period the slices were killed and the tissue sucrose extracted.

sucrose produced remained in the slices. Crops of slices giving low amounts of sucrose leakage were frequently encountered. The initial rate of leakage from such slices was high (*ca.* 20 μmoles/hr g fr. wt.) and approximately equal to sucrose production, but after the first hr the rate of leakage declined so that the total leakage over the 2·5–3 hr experimental period was low (e.g. see Fig. 3) and the tissue sucrose level increased (Table 2). The newly synthesized sucrose that did not leak is assumed to be stored in the vacuoles although there is little evidence in support of this.

Cations and Sucrose Leakage

Some compounds which increased sucrose leakage are listed in Table 3; compounds which caused little or no increase in sucrose leakage are listed in Table 4. It is clear that a high pH alone is not enough to cause increased leakage although Tris was not effective at a low pH (Table 4). Compounds which increased leakage are able to form complexes with or

TABLE 3. COMPOUNDS WHICH INCREASED THE LEAKAGE OF SUCROSE FROM SCUTELLUM SLICES*

Bathing solution (fructose (0·1 M) + additions)	Sucrose (μmoles)		
	Leakage	Tissue change	Net production
+ Nil	25·6	34·8	60·4
+ Tris (pH 7·5)	51·7	0	51·7
+ Tris (pH 7·5)	52·3	2·8	55·1
+ Tris (pH 7·9)	40·8	13·9	54·7
+ Tris (pH 7·9)	47·4	6·9	54·3
+ Phosphate (pH 7·5)	46·8	18·0	64·8
+ Phosphate (pH 7·5)	57·1	9·7	66·8
+ HCl (0·01 M)	44·0	15·2	59·2
+ Glycylglycine (pH 7·5) + EDTA (0·002 M)	56·1	−4·2	51·9
+ Glycylglycine (pH 7·5) + ATP (0·01 M)	47·2	4·2	51·4

* The slices (1·0 g fr. wt.) were incubated in the bathing solutions at 30° for 2·5 hr. The buffers were present at a concentration of 0·06 M. At the end of the incubation, samples of the bathing solution were taken for sucrose analysis and the slices were killed and extracted.

displace cations. Tris could act either as a large cation in a displacement reaction or as a complexing agent.[6] Since Tris is not active at pH 4·7 (Table 4) but is active at pH 7·5 or 7·9 (Table 3) it appears to be acting by forming complexes with cations. Glycylglycine which by itself did not increase sucrose leakage (Table 4, Fig. 2) was added to both EDTA and ATP to maintain a pH at which these latter two compounds form strong complexes with cations. In the absence of glycylglycine, EDTA and ATP caused much smaller increases in sucrose leakage. ADP and AMP increased leakage but were less effective than ATP (unpublished results). Since an appreciable amount of ATP was hydrolyzed to ADP and AMP during the course of the experiment (unpublished results) quantitative comparisons among these three

[6] H. R. MAHLER, *Ann. N. Y. Acad. Sci.* **92**, 426 (1961).

TABLE 4. COMPOUNDS WHICH CAUSED LITTLE OR NO INCREASE IN THE LEAKAGE OF SUCROSE FROM SCUTELLUM SLICES*

Bathing solution (fructose (0·1 M) + additions)	Sucrose (μmoles) Leakage	Tissue change	Net production
+ Nil	23·2	38·9	62·1
+ Tris (pH 4·7)	23·2	37·5	60·7
+ Glycylglycine (pH 7·5)	25·8	27·8	53·6
+ Glycylglycine (pH 8·5)	22·2	18·1	40·3
+ Glycine (pH 8·6)	15·6	23·7	39·3
+ Borate (pH 7·4)	22·2	38·9	61·1
+ Borate (pH 8·5)	28·6	32·0	60·6

* See Footnote, Table 3.

TABLE 5. EFFECT OF DIVALENT CATIONS ON SUCROSE LEAKAGE*

Bathing solutions (buffer and fructose + additions)	Sucrose leaked (μmoles)
+ Nil	27·8
+ Mg^{2+} (0·01 M)	18·9
+ Mn^{2+} (0·01 M)	14·4
+ ATP (0·01 M)	46·4
+ ATP (0·01 M) + Mg^{2+} (0·01 M)	27·2
+ ATP (0·01 M) + Mn^{2+} (0·01 M)	20·8
+ ATP (0·01 M) + Ca^{2+} (0·01 M)	17·2

* The slices (1·0 g fr. wt.) were incubated in the above solutions at 30° for 2 hr. The buffer was glycylglycine (0·06 M, pH 7·5). The fructose concentration was 0·1 M.

nucleotides are of little value. However, these results support the idea that ATP was acting by forming complexes with cations and not as an energy source. The addition of Mg^{2+}, Mn^{2+}, or Ca^{2+} completely blocked the effect of ATP on sucrose leakage and, except for the ATP–Mg^{2+} experiment, depressed the leakage below that obtained in fructose alone (Table 5). The inhibition of sucrose leakage by cations has been reported previously.[7]

To determine which tissue cation(s) was responsible for the inhibition of leakage, scutellum slices were incubated in HCl (0·01 M) and then the bathing solution was assayed for the cations listed in Table 6. Only Mg^{2+} and Ca^{2+} were removed in significant amounts by HCl, and it appears that one or both of these cations are responsible for blocking leakage. The effect of prior incubation in water on the amounts of Ca^{2+} and Mg^{2+} removed from the

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

slices by HCl is shown in Table 7. The water treatment had no effect on the amount of Ca^{2+} removed by HCl, but strongly depressed the amount of Mg^{2+} removed. These results suggest that during the water treatment Mg^{2+} was removed from contact with the bathing solution either by transport into the cell or, if it resides in the vascular elements, by being isolated within a plugged element. Mg^{2+} may form part of the substance making up the plug or it may be necessary for the synthesis of the plugging material. The latter suggestion would pertain to callose whose deposition at the sieve plates of the mature sieve tube is perhaps the result of extracellular synthesis.[8]

TABLE 6. CATIONS REMOVED FROM SCUTELLUM SLICES BY WATER OR HCl*

Cation	Amount (μmoles) removed from 1·0 g slices by:	
	H_2O	HCl
Mg^{2+}	1·20	7·95
Ca^{2+}	0·25	0·75
Cu^{2+}	< 0·05	< 0·05
Fe^{3+}	< 0·05	< 0·05
Mn^{2+}	< 0·05	< 0·05
Zn^{2+}	< 0·05	< 0·05

* The slices (1·0 g fr. wt.) were incubated in water or HCl (0·01M) for 100 min at 30°. At the end of this period, the bathing solution was collected for cation analysis.

TABLE 7. EFFECT OF PRIOR TREATMENT IN WATER ON THE AMOUNT OF Ca^{2+} AND Mg^{2+} REMOVED FROM SCUTELLUM SLICES BY HCl

Treatment	Amounts (μmoles) removed from 1·0 g slices	
	Ca^{2+}	Mg^{2+}
A 2·5 hr in HCl (0·01 M)	1·0	12·1
B 3 hr in H_2O followed by:	0·3	1·8
2·5 hr in HCl (0·01 M)	1·0	3·4

DISCUSSION

The sites of action of the buffers, of HCl or of ATP in initiating or maintaining the sucrose leakage from sliced or whole scutella could be either at the plasma membranes of the individual cells or at the cut ends of the vascular bundles. Thus, removal of cations by these compounds might cause the plasma membranes to become leaky. The leakage process in this case would consist of free diffusion of sucrose through 'holes' in the membrane. Our studies on the hexose space of the maize scutellum have led us to conclude that there are

[8] D. H. NORTHCOTE, *Essay. Biochem.* **5**, 89 (1969).

pores in the plasma membranes through which hexoses (but not sucrose) can freely diffuse.[3,9] It may be that the size of the pore is controlled by divalent cations as has been shown for cells of *Necturus* kidney.[10] On the other hand, the presence of the compounds listed in Table 3 might prevent (again, presumably by removal of cations) the formation of proteinaceous 'slime' or callose plugs in the sieve tubes or they might prevent the sealing off of the sieve tube protoplast at or near the cut end. The leakage process in this case would consist of vein loading and phloem transport with the bathing solution serving as the sink.

In this paper we have stressed the idea that sucrose leakage occurs through vascular tissues. Three kinds of evidence favor this idea: (1) the whole scutellum leaked appreciable amounts of sucrose only after the root–shoot axis was removed (Table 1, Fig. 7); (2) the leakage process was a labile one (Fig. 6, Table 2) and (3) there was a sucrose pool that could be emptied by leakage into Tris but whose level was maintained in the presence of Tris and fructose (Figs. 1, 4 and 5).

The leakage of sucrose (into Tris–fructose) upon removal of the root–shoot axis clearly points to the vascular system as the leakage channel in the whole scutellum. Furthermore, the same tissue appears to be operative in leakage from both the whole scutellum and the slices; since Tris or HCl increased leakage in both and in both the leakage process was a labile one. Sieve elements are quite sensitive to wounding which results in callose and slime plug formation, plastid breakdown, and failure of the protoplast to plasmolyze.[11–13] Currier *et al.*[12] compared 0·3 M sucrose and tap water as sectioning, storing and mounting fluids in the preparation of stem sections for plasmolytic studies of phloem. In sections prepared in tap water, the sieve tube plastids broke down and the protoplast failed to plasmolyze within 15 min after sectioning. In contrast, in sections prepared in 0·3 M sucrose the sieve tubes contained intact plastids and their protoplasts were plasmolyzable even after 2·5 hr. The results of Fig. 6 show that the leakage process in the scutellum slices was preserved to a greater extent in 0·1 M fructose than it was in water. Prior incubation in 1·0 M sorbitol was even more effective in protecting the leakage process (unpublished results). The use of ice water during the preparation and incubation of whole scutella or slices probably protected the leakage process by inhibiting the wound reactions initiated upon severing the root–shoot axis from the scutellum or upon slicing the scutellum.

The data of Figs. 1, 4 and 5 support the idea of a pool of leakable sucrose. Previous studies[1,2] also support this idea and, in addition, produced evidence that the cells containing the sucrose that makes up the leakable pool are separate from those in which most of the sucrose is synthesized. In these previous studies high fructose concentrations (up to 1·0 M) were used in the absence or presence of Tris or HCl; high concentrations of fructose inhibit the transport of newly synthesized sucrose and some sucrose, therefore, remains in the synthesis compartment of the cell.[7,14] Tris and HCl, although they caused an increase in sucrose leakage, had little effect on the amount that remained in the synthesis compartment; furthermore, when the transport of newly synthesized sucrose was completely inhibited by 1·0 M fructose plus 0·01 M HCl, sucrose leakage was only a little greater than leakage into HCl alone.[1] Clearly, these results support the idea of a leakable sucrose pool contained in a specialized group of cells within the scutellum slice.

[9] L. A. Garrard and T. E. Humphreys, *Nature* **207**, 1095 (1965).
[10] G. Wittembury, N. Sugino and A. K. Solomon, *Nature* **187**, 699 (1960).
[11] K. Esau, *Botan. Rev.* **16**, 67 (1950).
[12] H. B. Currier, K. Esau and V. I. Cheadle, *Am. J. Botan.* **42**, 68 (1955).
[13] E. M. Engleman, *Ann. Bot. N. S.* **29**, 83 (1965).
[14] T. E. Humphreys and L. A. Garrard, *Phytochem.* **5**, 653 (1966).

The data, then, are not consistent with the idea that Tris, HCl or the other compounds of Table 3 cause the majority of the cells to leak sucrose either through 'holes' or via sucrose transport mechanisms in the plasma membranes. There are, however, at least two other situations that would be consistent with this data. First, leakage occurs by free diffusion through holes in faulty plasma membranes. However, only relatively few cells have faulty membranes and the rate of leakage is limited by the rate of sucrose transport to these cells. The leakable pool would consist, therefore, of the sucrose contents of these faulty cells (incubation in Tris alone, Fig. 1) plus newly synthesized sucrose which is transported to these faulty cells (incubation in Tris plus fructose, Figs. 4 and 5). Transport to the faulty cells is inhibited by high fructose concentrations.[1,2] During incubation in water the faulty membranes are repaired as a result of redistribution of Mg^{2+} and Ca^{2+} and leakage is depressed (Fig. 6). Alternatively, leakage is the result of phloem transport of sucrose to the bathing solution sink. The leakable pool would consist of the sucrose contents of the phloem tissue (incubation in Tris alone, Fig. 1) plus newly synthesized sucrose which is transported to the phloem cells (incubation in Tris plus fructose, Figs. 4 and 5). Transport to the phloem cells is inhibited by high fructose concentrations so that, despite the presence of Tris or HCl, sucrose builds up in the synthesis compartments of the individual mesophyll parenchyma cells.[1,2] Incubation in water causes plugs to form in the phloem cells and leakage is depressed (Fig. 6).

We favor the second hypothetical situation involving phloem tissue since (a) little leakage occurred from the whole scutellum until the root–shoot axis was removed (Table 1) and (b) if leakage occurs by diffusion through holes in the membranes, then sucrose should also be taken up through these holes. However, Tris completely abolished and HCl strongly inhibited the uptake of exogenous sucrose.[1,2]

It is not unreasonable to assume that the scutellum slices have a functional phloem system. Currier *et al.*[12] used tissue slices 55 μ in thickness (only about one-tenth the thickness of the scutellum slices used herein) from a number of species, and showed that the mature sieve tube protoplasts could be plasmolyzed and deplasmolyzed in repeated cycles. The sieve tubes appeared, therefore, not to have been grossly injured by the sectioning procedures.

Allowing the idea of functional sieve tubes in the scutellum slices, the problem of how sucrose moves from the severed sieve element to the bathing solution still remains. When a sieve element is severed does the lumen remain open allowing mass flow of solution through the cut end or does the severed protoplast seal itself off (because the phloem is under pressure this could probably only occur at the sieve plate) by forming new plasma membrane through which sucrose and other solutes must pass during leakage? Our knowledge of the structure of the sieve tube is insufficient to answer these questions.[15] Exudation following a phloem incision in trees appears to be a mass flow. In most trees there is only a momentary flow of exudate although in some species (e.g. *Fraxinus americana*) the exudation may continue for hours or days.[16] Leonard and Glenn[17] exposed detached bean leaves to $^{14}CO_2$ and found ^{14}C-sucrose (but not ^{14}C-glucose or ^{14}C-fructose) in the water bathing the petiole. This leakage continued for at least 24 hr and was considered to originate in the phloem. In contrast, Hartt *et al.*,[18] in a similar experiment, found only a trace of ^{14}C in the water

[15] P. E. WEATHERLEY and R. P. C. JOHNSON, *Int. Rev. Cytol.* **24**, 149 (1968).
[16] M. H. ZIMMERMAN, *Ann. Rev. Plant Physiol.* **11**, 167 (1960).
[17] C. A. LEONARD and R. K. GLENN, *Plant Physiol.* **43**, 1380 (1968).
[18] C. E. HARTT, H. P. KORTSCHAK and G. O. BURR, *Plant Physiol.* **39**, 15 (1964).

bathing the cut ends of sugarcane leaves even though about two-thirds of the ^{14}C fixed moved from the portion of the leaf exposed to $^{14}CO_2$ to more basal portions including that part of the leaf just above the cut surface. The extent and duration of phloem exudation is probably controlled by the wound reactions which result in the formation of phloem plugs, and we suggest that the compounds listed in Table 3 inhibit phloem plug formation in the scutellum.

EXPERIMENTAL

Plant Material

Maize grains (*Zea mays* L., var. Funks G-76) were soaked in running tap H_2O 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 (in some cases, as noted, longitudinally) into slices $\leq 0{\cdot}5$ mm thick. The slices were washed in H_2O until the washings remained clear, blotted on filter paper and weighed in groups of 1·0 g. In preparing the whole scutella, the endosperm was carefully removed and the seedlings were washed in H_2O and blotted on filter paper. Excision was accomplished by making a single cut at the scutellar node. Each excised scutellum was placed in the appropriate bathing solution as it was cut. When whole scutella with root–shoot axes attached were used, they were placed in test tubes (one seedling per tube) in such a way that the scutellum (abaxial surface down) was submerged in the bathing solution (1·0 ml) and the root and shoot were bent upward along the wall of the tube out of contact with the solution. The solution covered the scutellar node.

Experimental Procedure

Detailed procedures for these experiments are given in the Tables and Figures. Each group of slices (1·0 g fr. wt.) was placed in a 25 ml flask containing 10·0 ml of the appropriate bathing solution. The slices were incubated at 30° in a "Gyrotory" water bath (New Brunswick Scientific Company, New Brunswick, N.J., U.S.A.). Procedures for the determination of sucrose leakage and for the preparation of the EtOH extracts of the tissue slices have been described previously.[7]

Carbohydrate Analysis

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.

Cation Analysis

Cation concentrations were determined by atomic absorption spectroscopy. The analyses were performed by Dr. H. L. Breland of the Soils Department, University of Florida.

Acknowledgement—We thank Mr. Donald Winsor and Mr. Terence Quinn for their valuable assistance.