Phytochemistry, 1972. Vol .11, pp. 1311 to 1320. Pergamon Press. Printed in England.

# SUCROSE LEAKAGE FROM EXCISED MAIZE SCUTELLA*

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(*Received* 24 *May* 1971, *in revised form* 7 *September* 1971)

**Abstract**—Sucrose leaked from the maize scutellum into water upon removal of the seedling axis. A greater amount of sucrose leakage occurred when the seedlings were incubated with their scutella in fructose solutions for a number of hr before removal of the axis. Leakage into water was more rapid than into mannitol, and as the mannitol concentration was increased the leakage rate decreased. $Ca^{2+}$ inhibited leakage when present in the bathing solution during the leakage period, but had little or no effect when it was added to the solution bathing the scutellum before the seedling axis was removed. At 30°, the leakage rate, initially high, declined rapidly; and leakage stopped within 90 min. At 1°, leakage was slower but the rate was maintained for at least 4 hr so that the total leakage was greater at 1° than at 30°. If scutella were kept at 30° until leakage stopped and then were placed at 1°, leakage resumed but at a rate lower than that from scutella excised directly into 1° water. The $Q_{10}$ of the sucrose leakage process was well below 2·0, and leakage was not inhibited by DNP or by anoxia. It is concluded that sucrose leakage in the scutellum occurs by a pressure flow of solution which exits from the tissue through the cut ends of the phloem. Moreover, evidence is presented that indicates the flow is not restricted to the phloem but extends in an uninterrupted stream through the cytoplasm of most or all living cells of the scutellum.

## INTRODUCTION

GARRARD and Humphreys proposed that sucrose leakage from slices of the maize scutellum occurs through cut ends of phloem in a pressure flow of solution.[1-3] This solution, made up largely of sucrose but also containing other organic materials and inorganic ions, was believed to originate in the mesophyll parenchyma cells, and to flow from these cells through the phloem and into the bathing solution. This proposal places the cytoplasm of most or all living scutellum cells in the transport stream. Therefore, it excludes vein loading and implies that long distance sugar transport is a physical process. The requirements for metabolic energy in this transport scheme are limited to sucrose synthesis, to sucrose transport across the tonoplast and to maintenance of the transport pathway.

This paper deals with sucrose leakage from the whole scutellum. It is shown that leakage has a low $Q_{10}$ and is not inhibited by $N_2$ atmosphere or by DNP. It is concluded that sucrose transport in the scutellum takes place in a pressure flow of solution. This conclusion is in agreement with findings obtained using scutellum slices which were reported in a previous paper.[3]

## RESULTS

### *Leakage and Temperature*

In these experiments, the seedlings (endosperm removed) were placed with the scutella immersed in fructose solutions at room temperature (22–24°). After 3 hr the scutella were excised into flasks containing water at 1, 4, or 30°, and sucrose leakage was followed with

* Florida Agricultural Experiment Station Journal Series No. 3955.

[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.* **10**, 2891 (1971).

time. It was shown before that little or no leakage occurs until the root–shoot axis is removed.[3] Paper chromatography of the bathing solutions after 3 hr of leakage revealed strong sucrose and fructose spots but only a faint glucose spot; when water was used in the initial incubation, instead of a fructose solution, a strong sucrose spot and faint spots for fructose and glucose were obtained.

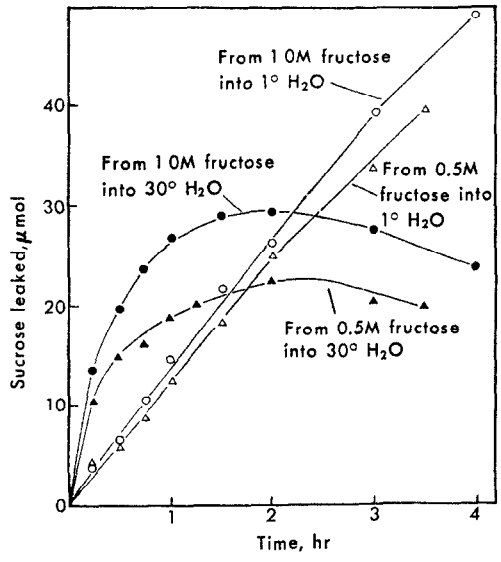


FIG. 1. SUCROSE LEAKAGE FROM SCUTELLA FOLLOWING TREATMENT IN 0·5 M OR 1·0 M FRUCTOSE. Seedlings were treated in fructose for 3 hr and then the scutella were excised (time zero) into flasks containing 1 or 30° water. Each flask contained 12 scutella (*ca.* 1·2 g fresh wt total tissue).

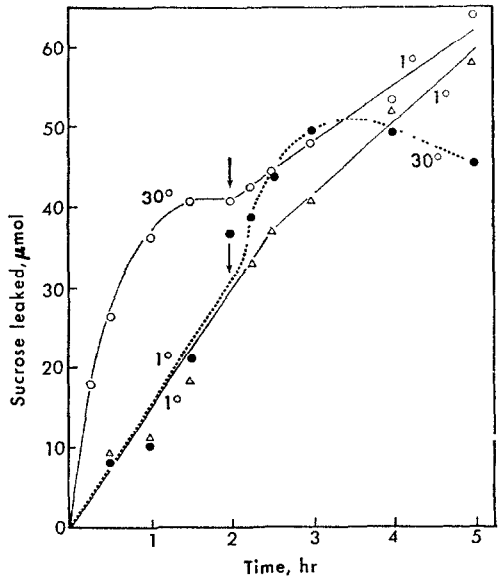


FIG. 2. RESUMPTION OF SUCROSE LEAKAGE UPON LOWERING THE TEMPERATURE FROM 30 to 1° See footnote, Fig. 1. The seedlings were treated in 1·0 M fructose. At the arrows the temperature was changed in two of the flasks.

Higher rates of sucrose leakage were found at 30° than at 1° (Fig. 1). However, the rates at 1° were considerable and were maintained for at least 4 hr so that the total amounts of sucrose leaked were greater at this temperature. The rates of leakage declined with time at 30° and after 2 hr the leakage stopped. Leakage from 0·5 M fructose-treated scutella declined much more rapidly than leakage from 1·0 M fructose-treated scutella. In contrast, scutella from both fructose treatments leaked sucrose at about the same rate when held at 1°. Assuming, on the basis of previous work,[1–3] that sucrose leakage results from a pressure flow of solution from severed phloem tissue, the results of Fig. 1 can be interpreted as reflecting the development at 30° of increased resistance to flow (phloem plugs or constrictions) which increases the threshold turgor pressure necessary for flow to occur. Since scutellum cells treated with 0·5 M fructose presumably have a lower turgor pressure than those treated in 1·0 M fructose, the rate of leakage declines more rapidly as resistance to flow develops at 30°.

The results of Fig. 2 are in agreement with this interpretation. In this experiment, the scutella were transferred to 1° after a 2-hr period at 30°. Before transfer, leakage had stopped; but it started again immediately after transfer to 1° and at a rate 75% as great as

the 1° control. When scutella were transferred to 30° after an initial 2-hr leakage at 1°, the rate of leakage sharply increased, then declined; and leakage stopped about 90 min after the transfer to 30°. It appears, therefore, that the resistance to flow developed at 30° can be partially reversed at 1°.

TABLE 1. $Q_{10}$ OF THE SUCROSE LEAKAGE PROCESS*

| Fructose conc. (M) | Temp. (°C) | Leakage rate, μmol/hr | $Q_{10}$ |
|---|---|---|---|
| 0·1 | 1 | 6·7 | |
| | | | 1·7 |
| 0·1 | 4 | 7·8 | |
| | | | 1·2 |
| 0·1 | 25 | 10·8 | |
| | | | 1·3 |
| 0·1 | 30 | 12·2 | |
| 1·0 | 1 | 14·8 | |
| | | | 1·5 |
| 1·0 | 4 | 16·6 | |
| | | | 1·6 |
| 1·0 | 30 | 55·3 | |

* The seedlings (minus endosperm) were incubated with their scutella in fructose at the concentrations indicated above. At the end of 3 hr, the scutella were excised and placed in flasks (12 per flask) containing water at the temperatures indicated. At the lower temperatures, the leakage rates were constant for 1 or more hr; at 25° and 30°, the leakage rates declined rapidly with time, and the rates given in the table were calculated from the values obtained during the first 15 min of leakage.

The temperature coefficients ($Q_{10}$) of the sucrose leakage process are shown in Table 1. The values in the table are from a single experiment for each fructose concentration. It is clear that the coefficients are well below 2·0 which suggests that leakage is a physical process. Viscous flow of a 20% aq. sucrose solution has a $Q_{10}$ of 1·3–1·4.[4]

The inhibitions of sucrose leakage at 1° by mannitol solutions are shown in Fig. 3. Hexoses and sugar alcohols readily move into and out of scutellum slices, apparently by free diffusion.[5] Movement of glucose out of (and presumably into) the slices is very slow at 1°.[5] Assuming that the movement of mannitol also is inhibited at 1°, the results of Fig. 3 can be taken to mean that mannitol decreased the turgor pressure driving the mass flow of sucrose solution. Moreover, as mannitol slowly entered the scutellum at 1°, the resulting increase in turgor pressure may have been offset by the increase in viscosity. Both turgor pressure and viscosity probably are factors in the mannitol inhibition of leakage.

*Evidence for the Involvement of Vascular Tissue*

Previously, it was shown that scutella treated in 1·0 M fructose leaked little or no sucrose

[4] N. A. LANGE, *Handbook of Chemistry*, McGraw-Hill, New York (1961).
[5] L. A. GARRARD and T. E. HUMPHREYS, *Nature, Lond.* **207**, 1095 (1965).

at room temperature until the root–shoot axis was removed, and these results were used as evidence for the involvement of vascular tissue in the leakage process.[2,3]

During the present study, it was found that also at 4° no leakage occurred until the root–shoot axis was removed (Fig. 4). In this experiment, the scutella were placed in 4° water (with or without the root–shoot axis attached) immediately after the endosperm was removed; therefore, sucrose leakage was at a lower rate than that from scutella which had been incubated 3 hr in fructose before excision (Figs. 1 and 3). It can be concluded that sucrose leakage resulted from removal of the seedling axis and not from an increased permeability of the plasma membrane towards sucrose at low temperatures. Thus, the idea that vascular tissue is involved in sucrose leakage is supported by the experiment of Fig. 4; furthermore, the facts that the leakage process is labile and is protected at low temperatures (Figs. 1 and 2; also see Ref. 2) suggest the phloem as the leakage conduit.

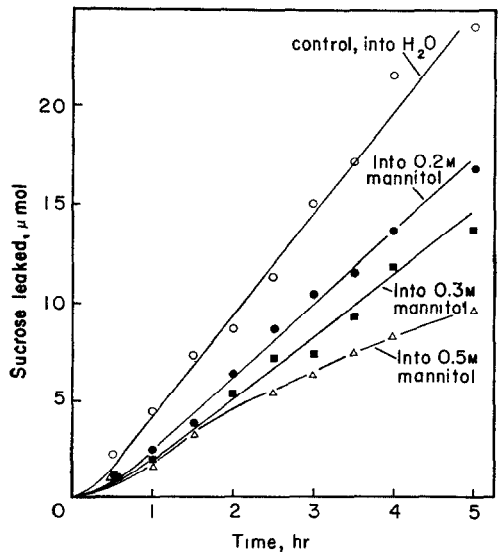


FIG. 3. EFFECT OF MANNITOL ON SUCROSE LEAKAGE. Seedlings were treated in 0·1 M fructose for 3 hr and then the scutella were excised (time zero) into 1° water or 1° mannitol at the concentrations shown. Each flask contained 12 scutella.

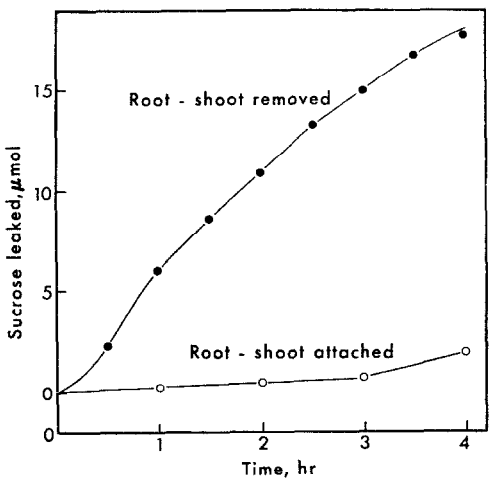


FIG. 4. EFFECT OF THE PRESENCE OF THE ROOT-SHOOT AXIS ON SUCROSE LEAKAGE AT 4°. Root–shoot axes were removed from 12 seedlings and the scutella were placed in 10 ml of 4° water. Sucrose leakage was measured every 30 min for 4 hr (upper curve). Twenty-four seedlings (endosperm removed) were placed individually in tubes with their scutella in 1·0 ml of 4° water (see Experimental). At the end of each hr, 6 tubes were collected, the seedlings were discarded and the solutions combined. The sucrose content of the combined solutions was multiplied by 2 to give leakage per 12 scutella (lower curve).

Additional evidence supporting vascular involvement in sucrose leakage comes from experiments with $Ca^{2+}$. When scutella were excised into $CaCl_2$ (0·1 M, 4°) instead of water, sucrose leakage was inhibited (Fig. 5). The $Ca^{2+}$ inhibition was almost complete following incubation of seedlings in 0·1 M fructose, but was only about 60% following incubation of seedlings in 1·0 M fructose. Although the seedlings were grown in tap water (about 2 ×

$10^{-3}$ M $Ca^{2+}$) it might be argued that sucrose leakage took place through plasma membranes which were 'leaky' as a result of $Ca^{2+}$ deficiency. The results of Fig. 6 show that the $Ca^{2+}$ effect was not on the plasma membranes. In this experiment, the seedlings were placed with the scutella in fructose (0·1 M or 0·5 M) with or without 0·1 M $CaCl_2$. After 3 hr in these solutions, the scutella were excised into 4° water and sucrose leakage was followed. The presence of $Ca^{2+}$ in the fructose solution had no effect on the subsequent leakage when 0·5 M fructose was used (high turgor pressure) and caused a significant decrease in leakage when 0·1 M fructose was used (low turgor pressure) only after about 2 hr following excision. The small inhibition of sucrose leakage from scutella treated in 0·1 M fructose plus 0·1 M $CaCl_2$ might well have been the result of $Ca^{2+}$ leaking from the tissue free-space and causing plugs to form in the phloem at the cut surface made when the root–shoot axis was removed.

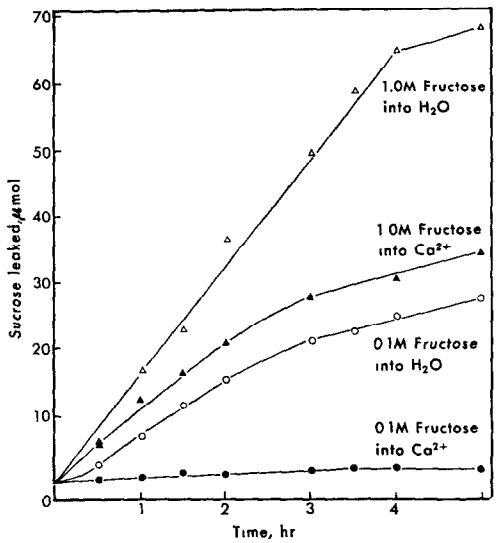


FIG. 5. EFFECT OF $Ca^{2+}$ ON SUCROSE LEAKAGE. See footnote, Fig. 1. Leakage was into 4° $CaCl_2$ (0·1 M) or 4° water.

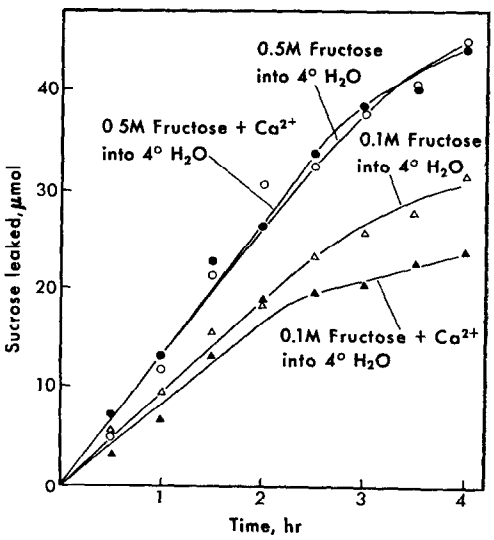


FIG. 6. EFFECT OF PRIOR $Ca^{2+}$ TREATMENT ON SUCROSE LEAKAGE. Seedlings were treated with fructose or fructose plus 0·1 M $CaCl_2$ for 3 hr at 24°. Then the seedlings were washed two times with water and the scutella were excised into 4° water. Each flask contained 12 scutella.

### *Effects of Dinitrophenol and a* $N_2$ *Atmosphere on Leakage*

The low, temperature coefficients of the leakage process (Table 1) indicate that leakage is a physical process. The fact that leakage was not inhibited by DNP supports this conclusion (Fig. 7). In this experiment, the seedlings were subjected to a 3-hr fructose (1·0 M) treatment followed by 1 hr in water or DNP before the scutella were excised into water at 1°. The fructose and DNP (or water) treatments were at room temperature so that penetration and action of DNP was not inhibited by low temperature. The 1-hr treatment in water (or DNP) following the fructose incubation caused about a 30% decrease in the leakage rate (compare Fig. 1 with Fig. 7). This was probably the result of a loss of fructose (by leakage through the plasma membranes and by metabolic utilization) which lowered the turgor pressure driving the mass flow of solution.

$N_2$ bubbled through the bathing solution during the 4-hr leakage period also did not inhibit sucrose leakage at 1 or 24°. In the $N_2$ experiments the seedlings were placed in air with their scutella immersed in 1·0 M fructose for 4 hr before the leakage period in $N_2$.

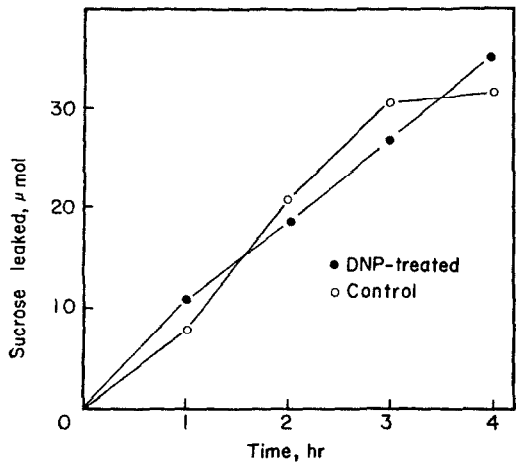


FIG. 7. EFFECT OF DNP ON SUCROSE LEAKAGE. Seedlings were treated in 1·0 M fructose for 3 hr at 24°. Then the fructose was removed and the seedlings were washed and treated in water or DNP ($5 \times 10^{-4}$ M) for 1 hr at 24°. At the end of the second treatment the scutella were excised (time zero) into 1° water. Each flask contained 12 scutella.

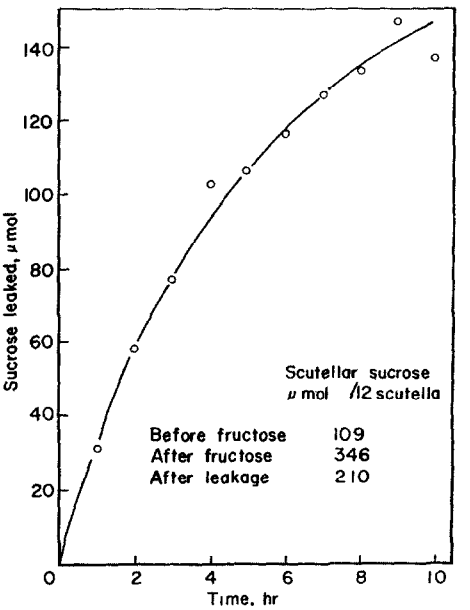


FIG. 8. ACCUMULATION OF LARGE QUANTITIES OF LEAKABLE SUCROSE IN THE SCUTELLUM. The seedlings were treated in 1·0 M fructose for 14 hr at 24°. The scutella were then excised into 1° water. Twelve scutella were extracted with ethanol before the fructose treatment, immediately after the fructose treatment and immediately after the 10 hr leakage period.

*Evidence that the Pressure Flow of Solution Originates in the Mesophyll Parenchyma Cells*

In a previous paper, evidence was presented that in scutellum slices the sources of leakable sucrose are for the most part mesophyll parenchyma cells and that these cells make up part of the conduit for the pressure flow system.[3] A strong argument for the involvement of the mesophyll cells is that the large quantities of leakable sucrose could not be entirely contained in the vascular tissue. A cursory examination of photomicrographs of cross sections of the scutellum indicates that less than 10% of the scutellar volume is made up of vascular tissue (also, see Ref. 6). Assuming that 50% of the vascular bundle is phloem tissue,[7] 12 scutella (80% water, about 1·2 g fr. wt) would contain less than 0·05 ml phloem water.

The experiment in Fig. 8 illustrates this argument. During a 14-hr incubation of the 12 scutella (root–shoot axes attached) in 1·0 M fructose, the sucrose content increased from 109 μmol to 346 μmol; at least 145 μmol of this sucrose was leakable (Fig. 8). If the leakable

[6] G. S. AVERY, JR., *Bot. Gaz.* **80**, 1 (1930).
[7] D. R. GEIGER, M. A. SANDERS and D. A. CATALDO, *Plant Physiol.* **44**, 1657 (1969).

sucrose was entirely dissolved in the phloem water (including phloem parenchyma, companion cells and sieve tubes) a solution in excess of 3 M would be formed. Because of its high viscosity, the presence of such a concentrated solution (*ca* 100%, w/v) in the scutellum is unlikely. The viscosity of a 20% sucrose solution is increased 2·5 times upon lowering the temperature from 30 to 0° whereas with a 60% sucrose solution there is a 7-fold increase.[4] A 100% sucrose solution would have an even greater $Q_{10}$. Since the rate of viscous flow is inversely proportional to the viscosity, the results of Table 1 indicate that a sugar solution of about 20% concentration is flowing. However, since fructose was also present in these experiments, the sucrose concentration was undoubtedly less than 20%.

Sucrose leakage from slices is much more rapid than it is from the whole scutellum, and leakage is completed within 90 min. Since slices retained their ability to synthesize sucrose during three successive sucrose synthesis-sucrose leakage cycles, it was concluded that leakage (postulated to be a pressure flow of solution through the mesophyll and phloem cells) did not cause cell damage.[3] The results of the experiments with whole scutella allow the same conclusion (Table 2).

TABLE 2. GAS EXCHANGE AND SUCROSE PRODUCTION FOLLOWING A PRESSURE FLOW OF SOLUTION FROM THE MAIZE SCUTELLUM*

| Prior treatment of seedlings | Gas exchange (whole scutella) μmol/hr g | | | Sucrose production† (slices) μmol/g | | |
|---|---|---|---|---|---|---|
| | $O_2$ | $CO_2$ | R. Q. | Tissue change | Leakage | Total |
| $H_2O$ (control) | 17 | 37 | 2·2 | 82 | 18 | 100 |
| Fructose (0·1 M) | 15 | 35 | 2·3 | 69 | 17 | 86 |
| Fructose (1·0 M) | 19 | 48 | 2·5 | 36 | 44 | 80 |

* Whole seedlings (minus endosperm) were treated in water or fructose for 3 hr as described in Experimental. At the end of this period the root–shoot axes were removed and the scutella were placed in water at 4° for 2 hr during which a pressure-flow of solution occurred. At the end of the 2-hr period, the scutella were placed in Warburg vessels for gas exchange measurements or were sliced and placed in fructose (0·1 M for 3 hr) for sucrose production measurements. Both measurements were made at 30°.

† Initially the slices contained the following amounts of sucrose (μmol/g): water pretreatment, 42; 0·1 M fructose pretreatment, 72; 1·0 M fructose pretreatment 108.

In the experiments of Table 2, whole scutella (root–shoot attached) were incubated at 24° in water or in two concentrations of fructose. At the end of the incubation, the scutella were excised and placed in 4° water for 2 hr during which time sucrose leakage occurred. The 24° water-incubated scutella served as controls in these experiments since only small amounts of sucrose leaked from these scutella (e.g. Fig. 4) and since the pressure difference between the mesophyll cells and the bathing solution was smallest of the three groups.

The whole scutellum, since it is bulky and has a high respiration rate, has a high R.Q; a result, no doubt, of alcoholic fermentation (Table 2, Ref. 8). Prior treatment in 0·1 M fructose had little effect on gas exchange whereas treatment in 1·0 M fructose caused about a 30% increase in $CO_2$ production. The extra $CO_2$ was probably the result of increased

alcoholic fermentation caused by the higher fructose and sucrose contents of these scutella.[8]

Slices from each of the 3 groups of scutella synthesized sucrose at high rates; although, slices from fructose-treated scutella synthesized 15–20% less sucrose than the water controls. Slices from fructose-treated scutella contained much more sucrose than the water controls (footnote, Table 2) which may have limited production. During the period of sucrose synthesis when the slices were incubated in 0·1 M fructose, large quantities of sucrose leaked from slices prepared from 1·0 M fructose-treated scutella. The leakage channels were open in these slices perhaps as a result of the surge of solution that flowed through them before slicing (during the 2-hr leakage period following the excision of the scutella) or as a result of the high turgor pressure supported by the sugar content of these slices (footnote, Table 2).

## DISCUSSION

Two kinds of evidence indicate that sucrose leakage from the maize scutellum is a physical process: the temperature coefficients were well below 2 (Table 1) and leakage was not inhibited by DNP or by a $N_2$ atmosphere (Fig. 7). The results of Figs 4–6 (also Refs. 1–3) indicate that the vascular system is the leakage conduit. However from Fig. 8, it is clear that the amount of sucrose leakage was much too large for it to have come only from phloem tissue; therefore, the mesophyll parenchyma cells must be the source of most of the leakable sucrose.

The force causing the movement of sucrose could be simple diffusion or a pressure flow of solution. Certainly, when the scutella are excised into water, the cell contents are under a hydrostatic pressure whose magnitude depends to a great extent on the length of the prior fructose incubation and on the concentration of the fructose (i.e. on the amounts of sucrose and fructose in the cells). The fact that $Ca^{2+}$ almost completely inhibited sucrose leakage from 0·1 M fructose-treated scutella whereas it only partially inhibited leakage from 1·0 M fructose-treated scutella (Fig. 5) is taken to mean that $Ca^{2+}$ increases resistance to flow. In this instance, the resistance was increased to an extent that the turgor pressure of the 0·1 M fructose-treated scutella, but not of the 1·0 M fructose-treated scutella, was balanced and no flow occurred. The inhibition of leakage by mannitol (Fig. 3) is also in agreement with the pressure flow idea.

The above arguments suggest that a pressure flow of solution and not diffusion is the mechanism for leakage. Moreover, the rate of leakage appears to be too great to be accounted for by free diffusion. However, sucrose might diffuse into the phloem from the mesophyll cells and the pressure flow might be limited to the phloem conduit. If this were the case, the diffusion path would include the plasmodesmata; otherwise, sucrose leakage should not require the excision of the root–shoot axis but should diffuse from the cell wall free-space of the tissue. Leaching of carbohydrates from the leaves of many plants has been observed,[9] and Kursanov and Brovchenko suggest that the leaf free-space is an important pathway for the movement of carbohydrates from the mesophyll to the vascular bundle.[10] The results of Fig. 4 and Ref. 3 show that the freespace pathway is not used for sucrose transport in the maize scutellum at 4 or 25°.

The structure and function of the plasmodesma are poorly understood.[11] If it is an open

[8] L. A. GARRARD and T. E. HUMPHREYS, *Phytochem.* 7, 1949 (1968).
[9] H. TUKEY, JR., S. WITTWER and H. TUKEY, *Science* **126**, 120 (1957).
[10] A. L. KURSANOV and M. I. BROVCHENKO, *Can. J. Bot.* **48**, 1243 (1970).
[11] P. A. L. CLOWES and B. E. JUNIPER, *Plant Cells*, Blackwells, Oxford (1968).

pore, a mass flow of solution could move sucrose among the cells, especially under conditions of steep pressure gradients which must have occurred in these experiments. Diffusive transport of sucrose would be negligible under these conditions. For the diffusion idea (either free or facilitated), a membrane positioned at right angles to the long axis of the plasmodesma and separating its two adjacent cells is required to prevent mass flow. This membrane should be freely permeable to sucrose even at low temperatures. However, it was shown that the plasma membranes of the scutellum allow free diffusion of hexoses (but not of sucrose) at 30° whereas at 1° movement is greatly restricted.[5] Moreover, hexose phosphates also leak from the scutellum when the root-shoot axis is removed.[12] It is necessary, then, to ascribe unusual properties to this postulated membrane positioned across the plasmodesmal opening.

In view of the above considerations, it is concluded that sucrose transport in the scutellum is by a pressure flow of solution. The flow is not restricted to the phloem but is believed to extend in an uninterrupted stream through the cytoplasm of most of all living cells of the scutellum. Identical conclusions, based on different considerations, were arrived at from studies using scutellum slices.[3]

The studies of Tammes and coworkers[13–15] on exudation from wounded *Yucca flaccida* inflorescence stalks indicate that sieve tube transport is a physical process and relatively temperature insensitive whereas sucrose movement from the non-vascular tissue into the phloem, being temperature sensitive, is driven perhaps by metabolism. However, studies on phloem transport in petioles indicate that chilling-inhibition of transport is limited to the need for energy to maintain the structure of the translocation conduit.[16–18] This may be true in *Yucca* stalk segments, and chilling inhibition of exudation may result, for instance, from closure of the plasmodesmata and not from retardation of an active secretory process.

## 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 filter paper in the dark at 24–25° for 72 hr. The endosperm was carefully removed from each seedling. The seedlings were placed in ice water as they were prepared and were rinsed 3 times in ice water before use. Scutellum slices (Table 2) were prepared according to the procedure of Garrard and Humphreys.[1]

*Experimental procedure.* Detailed procedures are given in the Tables and Figures. Each seedling was placed in a plastic, round bottom, 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 shoot were bent upward along the wall of the tube. Enough treatment solution (usually fructose) was then added to reach but not cover the scutellar node. The tubes were placed in a specimen jar which was lined with moist paper and covered with aluminum foil. The jar was kept at *ca.* 23° during the treatment period. At the end of the treatment, the seedlings were quickly washed and the root–shoot axis was removed by making a single cut at the scutellar node. Scutella were placed in 25 ml flasks as they were cut. Flasks contained 10 ml of water or solution into which leakage occurred. Flasks placed at room temp. or 30° were shaken; flasks placed at 1 or 4° were not shaken. Samples of the bathing solution were removed for sucrose analysis at the times indicated in the Figures. Procedures for the determination of

[12] T. E. Humphreys, *Phytochem.* **11**, 541 (1972).
[13] P. M. L. Tammes and J. Van Die, *Acta Bot. Neerl.* **13**, 76 (1964).
[14] J. Van Die and P. M. L. Tames, *Kon. Ned. Akad. Wetensch. Proc. Ser. C. Biol. Med. Sci.* **69**, 648 (1966).
[15] P. M. L. Tammes, C. R. Vonk and J. Van Die, *Acta Bot. Neerl.* **18**, 224 (1969).
[16] C. A. Swanson and D. R. Geiger, *Plant Physiol.* **42**, 751 (1967).
[17] D. R. Geiger, *Ohio J. Sci.* **69**, 356 (1969).
[18] D. R. Geiger and S. A. Sovonick, *Plant Physiol.* **46**, 847 (1970).

sucrose leakage and for the preparation of ethanolic extracts of the scutella have been described.[19] 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.

*Acknowledgements*—I thank Mr. Terrence Quinn for his valuable technical assistance. I gratefully acknowledge the valuable discussions with Dr. Leon Garrard during the preparation of this manuscript.

[19] L. A. GARRARD and T. E. HUMPHREYS, *Phytochem.* **6**, 1985 (1967).

*Key Word Index—Zea mays*; Gramineae; maize; excised scutella; sucrose leakage; temperature effects; calcium effects.