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EFFECT OF HYDROGEN ION CONCENTRATION ON SUCROSE LEAKAGE FROM CORN SCUTELLUM SLICES: EVIDENCE FOR TWO KINDS OF SUCROSE POOL WITHIN THE SLICE*

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Abstract—Incubation of corn scutellum slices in HCl concentrations above 0·01 M caused a marked disruption of cell function as evidenced by the copious leakage of sucrose from the tissue, the loss of the tissue's capacity for the synthesis and storage of sucrose from exogenously supplied hexose and the failure of the tissue to store exogenously supplied sucrose. In contrast, treatment of the tissue in 0·01 M HCl both in the presence and the absence of fructose increased the leakage of sucrose without seriously impairing sucrose synthesis. In addition, pretreatment of the slices in 0·01 M HCl did not reduce sucrose synthesis and storage in the presence of exogenous fructose or inhibit the storage of exogenous sucrose. The leakage of sucrose into 0·01 M HCl or 0·01 M HCl plus fructose is not ascribed to a disruption of the plasmalemma but rather to the existence of two pools of stored sucrose within the tissue, one mobile in acid and the other immobile in acid. The location of these pools within the tissue and the movements of sucrose between pools and bathing solution are discussed in relation to previously conceived ideas of cellular compartmentation and observed anatomical features of the scutellum tissue.

INTRODUCTION

FROM our studies of the corn scutellum, we identified a sucrose synthesis and a sucrose storage compartment(s) in the scutellum cell.[1,2] We postulated that the synthesis compartment contains, in addition to the enzymes for sucrose synthesis, the glycolytic enzymes,[3] and that it is separated from the storage compartment by a membrane which is impermeable to hexoses but which contains a mechanism for the active transport of sucrose into storage.[4] Since we define 'stored sucrose' as sucrose which does not leak from the tissue slices into water, our use of the term 'storage compartment' has no cytological connotation.

We showed that tris buffer (pH 7·5) caused stored sucrose to leak from the tissue, and we interpreted this as an effect of tris on the storage compartment membranes of individual parenchymatous cells.[4] In contrast, tris did not cause leakage of sucrose accumulated in the synthesis compartment.[4] In this paper we present the results of our studies of sucrose synthesis, storage and leakage in the presence of HCl. Our results suggest that the tissue contains two sucrose pools, one present in the individual parenchymatous cells comprising the scutellar body and another present in cells of vascular tissue.

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RESULTS

Effect of H^+ on Leakage, Production and Storage of Sucrose

When slices of the corn scutellum are incubated in fructose solutions, sucrose leaks from the slices into the surrounding medium. This leakage of sucrose (which we formerly have called 'leakage A') appears to involve a specific transport mechanism which moves sucrose directly from an intracellular storage compartment to the cell exterior.[1] However, under certain conditions (i.e. incubation in tris buffer), sucrose leaks from the slices by processes which appear to be unrelated to the leakage A mechanism.[4] Inasmuch as the investigations reported here were conducted under conditions which left some doubt as to the nature of sucrose leakage (i.e. whether all, part or none of the sucrose leaked was by the leakage A mechanism), the terms 'leakage' or 'sucrose leakage' will be used in lieu of 'leakage A'. If the tissue slices are incubated in fructose concentrations of 0·1 M or lower, sucrose storage takes place as rapidly as sucrose synthesis and little leakage of sucrose occurs if the slices subsequently are placed in water.[1] However, at fructose concentrations above 0·1 M, sucrose synthesis proceeds at a faster rate than sucrose storage and some sucrose accumulates in the synthesis compartment of the cell. This sucrose leaks freely into water and is termed 'leakage B' sucrose.[1] It will be noted that by our definition all sucrose leakages from the tissue slices, with the exception of leakage B, represent losses of stored sucrose from the tissue. Leakage B, however, is the loss of newly synthesized sucrose which never has entered a storage pool.

The effects of the H^+ concentration of the bathing medium on sucrose leakage from slices incubated in 0·1 M fructose are shown in Fig. 1. It may be seen that the rate of sucrose

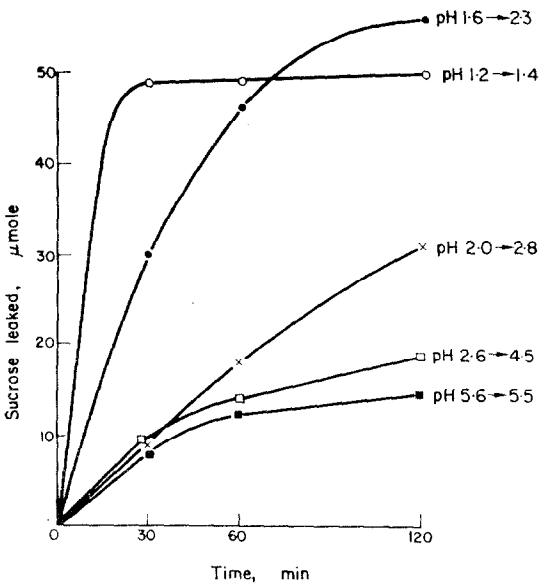

FIG. 1. THE EFFECT OF H^+ CONCENTRATION ON THE LEAKAGE OF SUCROSE FROM SCUTELLUM SLICES DURING INCUBATION IN FRUCTOSE.

The slices (1·0 g fr. wt.) were incubated in 0·1 M fructose to which enough HCl had been added to give the desired initial pH. The flask having an initial pH of 5·6 received no HCl. Incubation was at 30° for 2 hr during which aliquots of the bathing solution were taken at the times given above. These aliquots were analyzed for sucrose, and the final pH of the bathing solution was determined.

TABLE. 1. EFFECT OF H^+ ON LEAKAGE AND PRODUCTION OF SUCROSE*

pH		Sucrose (μmoles)			
		Leakage	Tissue level		Total produced
initial	after 2 hr		initial	after 2 hr	
1·2	1·4	50·0	56·9	0	−6·9
1·6	2·3	55·9	56·9	5·6	4·6
2·0	2·8	31·2	65·3	75·0	40·9
2·6	4·5	18·9	56·9	84·7	46·7
5·6	5·5	14·8	56·9	90·3	48·2

* The data above are from the same experiment shown in Fig. 1. After the 2-hr incubation period during which sucrose leakage was determined, the tissue slices were quickly rinsed with water and killed and extracted in hot ethanol. These extracts were used to determine tissue sucrose (in this case, stored sucrose). In addition, ethanolic extracts were prepared from untreated tissue slices for the determination of the initial sucrose content of the tissue. The sucrose data for slices with an initial pH of 2·0 were obtained from a separate experiment in which the slices had a higher initial sucrose content. The change in the amount of tissue sucrose during incubation plus the amount of sucrose leaked during incubation equals total sucrose production.

TABLE 2. THE EFFECT OF 0·01 M HCl ON SUCROSE STORAGE*

Bathing solution	Sucrose stored (μmoles)†	
	No pretreatment	
Sucrose (0·1 M)	38·0	
Sucrose (0·1 M)	41·7	
Sucrose (0·1 M) + HCl (0·01 M)	4·5	
Sucrose (0·1 M) + HCl (0·01 M)	11·1	
	Pretreated at 30° for 100 min in:	
	H_2O	HCl (0·01 M)
Sucrose (0·2 M)	43·8	45·1
Sucrose (0·2 M)	47·9	43·7
Fructose (0·1 M)	76·4	73·6
Fructose (0·1 M)	74·1	81·7

* Groups of slices (1·0 g fr. wt.) which were either freshly prepared or pretreated as shown above and washed in 10 ml of water were placed in the above bathing solutions for incubation at 30°. Slices receiving no pretreatment were incubated in sucrose for 3 hr while slices receiving pretreatment were incubated either in sucrose for 2 hr or fructose for 3 hr. Following incubations in sucrose, the tissue slices were washed in 10 ml of water and then placed in 10 ml of water for an additional 30-min incubation to remove leakable (unstored) sucrose. The tissue was then killed and extracted in hot ethanol for the determination of tissue contents of sucrose. In the case of fructose incubations, a portion of the bathing solution was taken at the end of the 3 hr period for the determination of sucrose leakage, and the tissue slices were killed and extracted for the determination of tissue (stored) sucrose. The leakage of sucrose during incubation in fructose was small in all cases (5–7 μmoles). The solutions containing HCl had an initial pH of 2·0.

† The values in the sucrose experiments are for net storage (final tissue sucrose content minus initial tissue sucrose content) while the values in the fructose experiments are for total sucrose storage (change in tissue content of sucrose plus sucrose leakage during incubation).

leakage increased as the pH of the fructose-containing bathing solution decreased. Table 1 gives additional data for the experiment shown in Fig. 1 and shows the completely disruptive effect on the tissue caused by incubation of the slices in solutions with initial pH values below 2·0. In these cases, the sucrose synthesis and storage capabilities of the tissue were destroyed. Such conditions would arise, presumably, from the destructive effect of high H^+ concentrations on the plasmalemma which in turn would lead to the exposure of the cell contents to harmful H^+ concentrations. In contrast, tissue slices treated in fructose with an initial pH of 2·0 or above leaked less sucrose and retained almost their full capacity for sucrose synthesis (Table 1). While tissue slices which were incubated at an initial pH of 2·0 leaked approximately twice the amount of sucrose as did slices incubated at an initial pH of 5·6, the synthesis of sucrose was reduced only about 15 per cent.

The storage of exogenous sucrose by scutellum slices was strongly inhibited when the bathing medium contained 0·01 M HCl (Table 2). However, pretreatment of the slices in 0·01 M HCl for 100 min at 30° had no effect on the storage of exogenous sucrose when the slices subsequently were incubated in the disaccharide. Clearly, pretreatment in HCl (pH 2·0) caused no irreversible change in the membranes associated with the storage of exogenous sucrose. The data of Table 2 also show that pretreatment in HCl (pH 2·0) did not affect the storage of newly synthesized sucrose when the tissue subsequently was incubated in fructose (0·1 M). It should be noted that since leakage B is negligible following incubation

TABLE 3. THE EFFECT OF PRETREATMENT OF SCUTELLUM SLICES IN HCl CONCENTRATIONS GREATER THAN 0·01 M ON SUCROSE SYNTHESIS AND STORAGE*

First incubation (HCl or H_2O for 1 hr at 30°)

Treatment	Initial pH	Final pH	Sucrose leaked (μmoles)	Tissue sucrose (μmoles)	
				initial	final
A (HCl)	1·70	2·15	55·3	71·4	10·3
B (HCl)	1·85	2·35	38·4	71·4	28·3
C (H_2O)	5·35	5·45	9·0	71·4	60·3

Second incubation (0·1 M fructose or 0·3 M sucrose for 3 hr at 30°)

Treatment	Sucrose leaked (μmoles)	Tissue sucrose (μmoles)			Sucrose, produced (μmoles)
		initial	final	change	
A (fructose)	9·2	10·3	9·2	−1·1	8·1
B (fructose)	11·8	28·3	41·4	13·1	24·9
C (fructose)	5·0	60·3	129·4	69·1	74·1
A (sucrose)	—	10·3	10·3	0	—
B (sucrose)	—	28·3	33·3	5·0	—
C (sucrose)	—	60·3	121·1	60·8	—

* Each treatment (A, B and C above) contained three groups of slices, each 1·0 g fr. wt. At the end of the first incubation, one group of slices from each treatment was used to determine tissue content of sucrose. The remaining two groups of slices from the treatment were then incubated either in fructose or sucrose as outlined above. Where applicable, sucrose leakage and tissue content of sucrose were determined (for procedure see footnotes Tables 1 and 2). Under conditions of this experiment, the sucrose production values for slices incubated in fructose are equal to sucrose storage values. In sucrose incubation experiments, the values for change in tissue sucrose are equal to net sucrose storage values.

in 0·1 M fructose,[1] the sucrose storage values for fructose incubations shown in Table 2 are equivalent to sucrose synthesis values. Thus, the inhibition of sucrose synthesis from exogenously supplied fructose (initial pH 2·0, see Table 1) occurred only if the HCl and fructose were used together.

Although pretreatment in 0·01 M HCl did not adversely affect the capability of the tissue to store exogenous sucrose and synthesize sucrose from fructose (Table 2), pretreatment of tissue slices in HCl solutions with pH values below 2·0 did cause a marked reduction in the storage of exogenous sucrose and the synthesis of sucrose from fructose when the slices subsequently were incubated in sugar solutions (Table 3). Thus, the changes in the tissue caused by high H^+ concentrations which resulted in the copious leakage of sucrose in the presence of 0·1 M fructose (Fig. 1, Table 1) and also during acid pretreatment (Table 3, first incubation) are irreversible changes, presumably, first of the plasmalemma and subsequently of other cellular constituents. Inasmuch as incubation of tissue slices in 0·01 M HCl (initial pH 2·0) increased sucrose leakiness without the disruptive effects on the cells cause by higher H+ concentrations, this concentration of HCl was used in further studies of sucrose leakage and storage.

Evidence for Two Pools of Stored Sucrose within the Tissue Slices

The leakages of sucrose with time from tissue slices incubated in water, 0·01 M HCl, 0·1 M fructose and 0·01 M HCl plus 0·1 M fructose are shown in Fig. 2. The amount of sucrose leakage into water was small, and the sucrose leaked was almost entirely reabsorbed by the end of the incubation period. Leakage into 0·1 M fructose was greater in amount

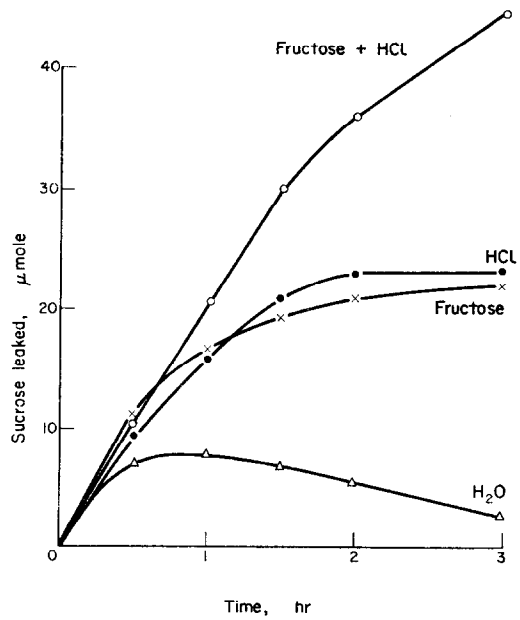

FIG. 2. THE EFFECT OF HCl ON THE LEAKAGE OF SUCROSE FROM SCUTELLUM SLICES INCUBATED IN THE PRESENCE AND ABSENCE OF FRUCTOSE.

The slices (1·0 g fr. wt.) were incubated in the solutions shown above (water, 0·01 M HCl, 0·1 M fructose or 0·1 M fructose plus 0·01 M HCl). Incubation was conducted at 30° for 3 hr and samples were taken at the times shown above for the determination of sucrose leakage.

than sucrose leakage into water and had reached a maximum value by the end of the incubation period. The sucrose leakage curve obtained in 0·1 M fructose was typical of curves obtained in previous studies.[1] The HCl curve for sucrose leakage is similar to that for fructose. In contrast to the sucrose leakage curve obtained with HCl alone, the curve obtained when slices were incubated in 0·01 M HCl plus 0·1 M fructose shows that sucrose continued to leak from the slices throughout the incubation period, and at the end of 3 hr, the amount of sucrose that leaked into HCl plus fructose was approximately double the amount that leaked into either HCl or fructose alone. Inasmuch as sucrose leakage into HCl alone represents a loss of stored sucrose from the slices (freshly prepared slices contain, by our definition, only stored sucrose), the question arises: Does the increased sucrose leakage into HCl plus fructose also represent solely sucrose leakage from storage or is the increase in leakage due to the exit of some newly synthesized sucrose from the synthesis compartment to the bathing solution?

The results of Table 4 clearly indicate that sucrose leakage into HCl plus fructose did not involve the movement of any newly synthesized sucrose from the synthesis compartment

TABLE 4. THE EFFECT OF H^+ ON THE PRODUCTION, STORAGE AND LEAKAGE OF SUCROSE*

Bathing solution†	Sucrose (μmoles)						
	Change in tissue	+	Leakage	=	Total storage	Leakage B	Total produced
Fructose (1·0 M)	18·1	+	13·6	=	31·7	55·2	86·9
Fructose (1·0 M)	20·9	+	12·3	=	33·2	48·4	81·6
Fructose (1·0 M)+HCl (0·01 M)	−31·9	+	34·5	=	2·6	42·5	45·1
Fructose (1·0 M)+HCl (0·01 M)	−31·9	+	27·6	=	−4·3	50·7	46·4

* The slices (1·0 g fr.wt.) were incubated in the bathing solutions for 3 hr at 30°. At the end of the 3-hr period, a portion of the bathing solution was removed for the determination of sucrose leakage, the slices were washed quickly with 10 ml of water and then 10 ml of water were added to the flask. Incubation was continued for an additional 90 min and then a portion of the bathing solution was removed for the determination of leakage B sucrose. The slices were then killed and extracted in hot ethanol for the determination of tissue (stored) sucrose. Change in tissue sucrose was calculated by subtracting the amount of sucrose found in the slices at zero time from those amounts found in the slices after the total incubation period (3 hr plus 90 min).

† The pH's at the beginning and at the end of the 3-hr incubation period were as follows: fructose, 5·4–5·5; fructose plus HCl, 2·0–3·2.

into the bathing medium. In this experiment, tissue slices were incubated in 1·0 M fructose (with or without 0·01 M HCl) in order to inhibit the movement of newly synthesized sucrose from the synthesis compartment to the storage compartment of the cell and thus allow it to accumulate within the synthesis compartment.[1] It may be seen that the addition of 0·01 M HCl to 1·0 M fructose (Table 4) inhibited sucrose synthesis by approximately 45 per cent, whereas 0·01 M HCl only inhibited sucrose synthesis by 15 per cent in the presence of 0·1 M fructose (Table 1). This increase in the inhibition of sucrose synthesis caused by 0·01 M HCl at the higher fructose concentration might be related to some stress condition arising in the tissue as a result of a high external sugar concentration. Note that in acid-treated slices, the amount of unstored sucrose (leakage B) was approximately the same as the amount of sucrose produced by the tissue, and that the amount of sucrose storage was negligible.

Also, the change in tissue sucrose (stored sucrose) in acid-treated slices was of the same magnitude as the amount of sucrose which leaked into the medium during the incubation. These observations strongly support the idea that although HCl causes the leakage of stored sucrose from the tissue slices, the newly synthesized sucrose of the synthesis compartment is separated from the cell exterior by a membrane which is resistant to the action of 0·01 M HCl.

Table 5 shows the effects of 0·01 M HCl on the leakage of stored sucrose in the presence and absence of fructose. When fresh slices (containing only stored sucrose) were incubated in 0·01 M HCl, there was a negative value for sucrose production because of a metabolic utilization of the disaccharide. Since leakage stopped after 2 hr (Fig. 2) and the tissue still contained sucrose (Table 5), it appears that the scutellum slices contain two pools of stored sucrose, one which leaks into acid (acid-mobile pool) and another which resists leakage into acid (acid-immobile pool). The results obtained using 1·0 M fructose plus 0·01 M HCl support this idea. Under these conditions, the newly synthesized sucrose remains in the synthesis compartment (Table 4). However, of the initial quantity of stored sucrose in the tissue, some leaked into the bathing solution and some remained in the tissue (as stored sucrose). Both the amounts of sucrose which leaked during incubation in 1·0 M fructose and 0·01 M HCl and the amounts that remained in the tissue after leakage B had occurred

TABLE 5. THE EFFECT OF HCl ON SUCROSE LEAKAGE FROM STORAGE: EVIDENCE FOR TWO SUCROSE POOLS*

Fructose conc. (M)	Time (hr)	Sucrose (μmoles)				Ratio†
		Total production	Leakage "B"	Tissue level after "B"	Leakage	
nil	3	−8·7	0	36·1	23·3	0·65
nil	3	−5·1	0	38·9	21·3	0·55
0·1	3	54·0	1·3	75·0	43·0	0·57
0·1	3	61·6	2·0	83·3	44·4	0·53
1·0	3	46·4	50·7	35·3	27·6	0·78
1·0	3	45·1	42·5	30·6	34·5	1·13
1·0	4	55·4	58·0	36·1	29·4	0·81

* The slices (1·0 g fr. wt.) were incubated at 30° in 0·01 M HCl or in 0·01 M HCl plus fructose at the concentrations shown. The procedures for the determination of sucrose leakage, leakage B and tissue sucrose are given in the footnote, Table 4.

† The ratio is equal to the amount of sucrose leaked into the bathing medium divided by the tissue level of sucrose following leakage B.

were similar to those found when the slices were incubated in HCl alone. In the case of incubation in 1·0 M fructose and 0·01 M HCl, the leakage of sucrose also had reached completion during the treatment period (compare 3-hr with 4-hr incubations, Table 5). It may also be seen in Table 5 that under conditions where the tissue rapidly synthesizes and stores sucrose (i.e. incubation in 0·1 M fructose plus 0·01 M HCl), the leakage of sucrose during incubation increased and the tissue level of sucrose following leakage B also increased. This indicates that the storage of newly synthesized sucrose involves movement of the disaccharide into both storage pools of the tissue.

The ratio values of Table 5 reflect the relative sizes of the two sucrose storage pools, that is to say, the amount of tissue sucrose after leakage B (acid-immobile pool) divided by the amount of sucrose leaked during incubation in acid (acid-mobile pool). The ratios obtained with 0·1 M fructose plus 0·01 M HCl were essentially the same as those obtained when the slices were incubated in 0·01 M HCl alone. This would mean that under conditions which do not inhibit sucrose storage the newly synthesized sucrose moves into both sucrose storage pools. Assuming that sucrose is leaking from the acid-mobile pool at the same rate that it is entering, the distribution of newly synthesized sucrose between the two pools is approximately equal. Incubation of tissue slices in 1·0 M fructose plus 0·01 M HCl resulted in higher and somewhat more variable ratio values. This probably was the result of an internal redistribution of sucrose caused by the high external fructose concentration.

TABLE 6. THE EFFECT OF PRETREATMENT IN WATER ON THE LEAKAGE OF SUCROSE INTO HCl AND HCl PLUS FRUCTOSE*

Second treatment, 2·5 hr	Sucrose leaked during second treatment (μmoles)	Tissue sucrose at end of second treatment (μmoles)
HCl, 0·01 M	0·4	38·9
HCl, 0·01 M + Fructose, 0·1 M	7·7	113·9

* Three groups of slices (each 1·0 g fr. wt.) were first incubated in water for 3 hr at 30°. At the end of the ncubation period in water, one group of slices was killed and extracted in hot ethanol for the determination of tissue sucrose and the other two groups of slices were placed either in HCl or HCl plus fructose as shown above and incubated for an additional 2·5 hr at 30°. Sucrose leakage was determined by analysis of a portion of the bathing medium, and tissue sucrose determined following hot ethanol extraction of the tissue slices. The tissue level of sucrose in the slices following their 3-hr incubation in water was 47·2 μmoles/g fr. wt.

If tissue slices were first incubated in water for 3 hr prior to being placed in 0·01 M HCl or 0·01 M HCl plus 0·1 M fructose, the amount of sucrose which leaked into the acid-containing bathing solution was greatly diminished (Table 6). Without pretreatment in water, the tissue slices leaked approximately 23 μmoles of sucrose during a 2·5-hr incubation in 0·01 M HCl and approximately 41 μmoles of sucrose during a 2·5-hr incubation in 0·01 M HCl plus 0·1 M fructose (Fig. 1); however, if the slices were pretreated in water for 3 hr, leakages into HCl and HCl plus fructose were reduced to 0·4 and 7·7 μmoles, respectively.

DISCUSSION

In previous studies of sugar interconversions and transport in the corn scutellum,[1–8] we have assumed that our results could be interpreted in terms of a typical parenchymatous cell within the scutellum body. This assumption was based on the fact that the majority of cells in the scutellum slices are parenchymatous. The results of the present study suggest that some observations made previously may not reflect responses of typical mesophyll parenchyma and that other cell types may be involved (e.g. vascular tissue).

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Although the scutellum consists primarily of parenchymatous cells, the abaxial surface of the organ is covered by a layer of columnar epithelial cells where the scutellum is in contact with the endosperm. A large vascular strand runs longitudinally down the center of the scutellum (parallel to the root–shoot axis) and is connected through the scutellar node with the vascular system of the seedling axis. The main vascular strand branches at a number of points giving rise to smaller vascular strands which also roughly parallel the root–shoot axis.[9] In preparing tissue slices (cut at right angles to the long axis of the scutellum) we cut across numerous vascular strands. This leaves within each slice vascular strand segments 0·5 mm or less in length which are 'open' at both ends.

Light and electron microscopy revealed that in the cells of the mesophyll parenchyma, the vacuolar system is disperse and occupies an appreciable cell volume. Furthermore, the degree of vacuolation of the parenchyma increases as the distance from the epithelial layer increases (i.e. cells near the axial surface contain more and larger vacuoles). In addition, the cells of the parenchyma and epithelial layer contain numerous primary cell wall pits with plasmadesmata connecting adjacent cells.[10]

During the present investigation, we have shown the deleterious effect of high H^+ concentrations on cells of the corn scutellum. At H^+ concentrations above 0·01 M, the tissue slices leaked sucrose copiously, and the cells lost their ability to synthesize and store sucrose during incubation in fructose as well as their ability to store exogenously supplied sucrose (Fig. 1, Tables 1 and 3). The changes in the tissue leading to these responses were not reversed by the removal of the acid solutions bathing the tissue slices (Table 3) and are considered, therefore, to constitute a massive disruption of cell structure and function. In contrast, treatment in 0·01 M HCl increased the leakiness of the tissue without seriously impairing the capacity of the tissue for sucrose synthesis (Table 1). Furthermore, after pretreatment with 0·01 M HCl, there was no impairment of either sucrose synthesis and storage or the storage of exogenous sucrose (Table 2). Inasmuch as sucrose synthesis was unimpaired by treatment with 0·01 M HCl, we conclude that the plasmalemma is an effective barrier to H^+ at this concentration. We do not, therefore, ascribe the leakage of sucrose in the presence of 0·01 M HCl or 0·01 M HCl plus fructose to a disruption of the plasmalemma but rather to the existence of two pools of stored sucrose within the tissue, one mobile in acid and the other immobile in acid. The results shown in Fig. 2 and Tables 4 and 5 support this idea. In considering the location of these two sucrose pools within the tissue, we find that at least two interpretations of our data are possible.

First, our results may be interpreted on the basis of a typical parenchymatous cell of the scutellar body which contains three compartments: (1) the sucrose synthesis compartment (metabolic compartment), (2) a cytoplasmic sucrose storage compartment and (3) a vacuolar storage compartment (the total volume of the disperse vacuolar system within the cell). If this basis for interpretation is correct, then both the synthesis compartment and the cytoplasmic storage compartment would be separated from the cell exterior by the plasmalemma, but only that portion of the plasmalemma bounding the cytoplasmic storage compartment would be sensitive to 0·01 M HCl. Thus sucrose within the cytoplasmic storage pool would be free to leak into 0·01 M HCl while stored sucrose of the vacuoles and newly synthesized sucrose accumulated in the synthesis compartment would not. In addition, incubation in water prior to acid treatment would modify the plasmalemma in such a way as to make it insensitive to subsequent acid treatment. Also, high fructose concentrations would inhibit

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[10] L. E. SAPP, Botany Department, University of Florida, Gainesville, Florida (1969).

sucrose movement from the synthesis compartment but not from the cytoplasmic storage compartment.

Alternatively, our results may be interpreted on the basis of individual scutellum slices each containing a comparatively small amount of vascular tissue (running at right angles to the plane of the slice) and a large amount of parenchymatous tissue the cells of which contain two kinds of compartments: (1) the cytoplasm or synthesis compartment (metabolic compartment) and (2) the vacuolar system (the storage compartment). In this scheme, the plasmalemma of the parenchyma cell would be insensitive to 0·01 M HCl (e.g. sucrose would not leak through the plasmalemma during incubation in HCl), and the sucrose which appears in the bathing solution during incubation in 0·01 M HCl or 0·01 M HCl plus 1·0 M fructose has as its origin the phloem tissue of the vascular strands. Presumably, the sieve tubes which are cut during the preparation of the slices are 'opened' and remain open in the presence of HCl. Thus, sucrose of the phloem (from Table 5 we presume this amounts to about 25 μmoles/g fr. wt. of slices) could move through sieve tubes into the bathing solution which acts as a sucrose sink. In a somewhat analogous situation, Leonard and Glenn[11] studying the vein loading of ^{14}C-assimilate in detached bean leaves found that the aqueous solution bathing the bases of petioles of detached leaves acted as a sink for ^{14}C-sucrose. Inasmuch as sucrose was the only sugar found in these solutions, it appears probable that the movement of assimilate out of the bean leaf petioles occurred through normal transport channels (e.g. sieve tubes). Thus, it also appears possible that the sieve tubes of scutellum slices could be the source of sucrose which appears in the surrounding medium during incubation in 0·01 M HCl or 0·01 M HCl plus 1·0 M fructose. Whether or not this movement of sucrose from the cut sieve tubes to the bathing solution constitutes active transport or passive diffusion is not known. The polarity of transport of sugars from leaf mesophyll cells to vascular tissue (phloem) is a well established fact, and it appears that the cells of the phloem tissue are capable of accumulating large amounts of leaf assimilate. For example, Bieleski[12,13] found that excised petiolar vascular bundles and excised phloem tissues of a number of plants could take up phosphate, sulfate and sucrose against high concentration ratios (true metabolically-linked accumulation). In addition, studies of the sites of accumulation in excised phloem and vascular tissue were made. Young secondary phloem was generally the most active tissue in regard to accumulation, and sieve tubes were among the most active, accumulating cells present.[13] We believe that in the corn scutellum, as in the case of photosynthetically active leaves, there is a polarity of sucrose transport from the mesophyll parenchyma to the phloem and an accumulation of the disaccharide in the sieve tubes. Thus, conditions which alter transport from the parenchyma to the phloem would also affect the amount of sucrose available for movement into the bathing solution. We observed that the amount of sucrose entering the bathing medium increased if 0·1 M fructose were used instead of 1·0 M fructose during incubations in 0·01 M HCl (Table 5, Fig. 2). In order to explain this observation, we suggest that high fructose concentrations (1·0 M) inhibit the movement of sucrose from the mesophyll parenchyma cells to the vascular strands and newly synthesized sucrose accumulates in the cytoplasm of parenchyma cells of the scutellum mesophyll. However, high fructose concentrations do not inhibit the movement of sucrose from the sieve tubes into the acid bathing medium. The inhibition of sucrose movement from mesophyll cells to phloem tissue could result from an initial shrink-

[11] O. A. LEONARD and R. K. GLENN, *Plant Physiol.* **43**, 1380 (1968).
[12] R. L. BIELESKI, *Plant Physiol.* **41**, 447 (1966).
[13] R. L. BIELESKI, *Plant Physiol.* **41**, 455 (1966).

ing of protoplasts in solutions of high osmotic concentration and a concomitant displacement of plasmadesmata, the normal transport channels between adjacent cells. We also suggest that the greater leakage of sucrose into 0·01 M HCl plus 0·1 M fructose is the result of some newly synthesized sucrose being transported from the mesophyll to the phloem tissues.

The leakage of sucrose into 0·01 M HCl and 0·01 M HCl plus 0·1 M fructose was greatly reduced if the tissue slices were first incubated in water for a period of 3 hr (Table 6). We have noted previously that sucrose leakage into nonacidic fructose solutions[7] (formerly termed leakage A) and sucrose leakage into tris buffer, pH 7·5 (unpublished data) were both reduced by a pretreatment of the tissue slices in water. It is possible that all the leakages described above originate at the same site, the open lumens of severed sieve tubes.

EXPERIMENTAL

Plant Material

Corn grains (*Zea mays* L., var 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.

Experimental Procedure

While the detailed procedures for these experiments are given in the tables and figures, certain methods remained the same throughout these investigations. Each group of slices (1·0 g fr. wt.) was placed in a 25 ml Erlenmeyer flask containing 10·0 ml of the appropriate bathing solution. Incubation of the slices was conducted at 30° in a "Gyrotory" water bath (New Brunswick Scientific Company, New Brunswick, N.J.). The handling of samples taken from the bathing solutions for the determination of sucrose leakage and the preparation of the ethanolic extracts of the tissue slices have been described previously.[2]

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 (Glucostat, Worthington Biochemical Corp., Freehold, N.J.).

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