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THE EFFECT OF TRIS(HYDROXYMETHYL)AMINOMETHANE ON SUCROSE STORAGE IN AND LEAKAGE FROM CORN SCUTELLUM SLICES*

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Abstract—Sucrose leaked from slices of the corn scutellum incubated in tris(hydroxymethyl)aminomethane (tris) buffer (0·06 M, pH 7·5). When the slices were incubated in tris buffer plus fructose the amount of sucrose leakage was increased. The sucrose content of slices incubated in tris buffer plus sucrose declined nearly as much as those incubated only in tris indicating that tris almost completely inhibited the storage of exogenous sucrose. Evidence is presented that these results were due to the action of tris on the membrane separating the storage compartment of the scutellum cell from the cell exterior rather than on the membrane between the sucrose synthesis compartment and the cell exterior. Slices pretreated in tris buffer for 1 hr and then quickly washed with water were no longer leaky, but the inhibition of exogenous sucrose storage persisted. This inhibition could be reversed by H^+ and either Al^{3+} or certain divalent cations.

INTRODUCTION

THE MOVEMENTS of sucrose in the corn scutellum cell as deduced from previous studies in this laboratory[1–4] are shown in Fig. 1. A single cell is depicted although slices of the scutellum were used in these studies. However, since the corn scutellum is composed of parenchyma except for small amounts of vascular tissue and an epithelial layer,[5] in what follows we interpret the results in terms of the individual parenchyma cell.

Glucose or fructose enters the sucrose synthesis compartment by free diffusion[1] and sucrose is rapidly synthesized and then transported (arrow 1) into the storage compartment.[2] The removal of sucrose from the storage compartment for metabolic utilization[2] occurs at a rate of 5–6 μmoles per hr per g fresh wt. and is represented by arrow 2. Arrow 2 points into the synthesis compartment and not into other cell regions because it is thought that the glycolytic system is located in the synthesis compartment.[3] The double arrow 3 represents an exchange of sucrose between the two compartments and no net movement is denoted.[2] At fructose concentrations above 0·1 M in the cell exterior, sucrose is not stored as rapidly as it is synthesized and sucrose accumulates in the synthesis compartment from which it leaks when the cell is placed in water.[2] This leakage is represented by arrow B. Arrow A represents leakage from the storage compartment which is accompanied by an exchange between the fructose moiety of the sucrose and the free fructose in the cell exterior.[2] Arrow 5

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[1] L. A. GARRARD and T. E. HUMPHREYS, *Nature* **207**, 1095 (1965).
[2] T. E. HUMPHREYS and L. A. GARRARD, *Phytochem.* **5**, 653 (1966).
[3] L. A. GARRARD and T. E. HUMPHREYS, *Phytochem.* **7**, 1949 (1968).
[4] T. E. HUMPHREYS and L. A. GARRARD, *Phytochem.* **7**, 701 (1968).
[5] H. E. HAYWARD, *The Structure of Economic Plants*, MacMillan, New York (1937).

also represents sucrose leakage from the storage compartment but, unlike leakage A, it can occur in the absence of external hexose and is increased by citrate–phosphate buffers.[4] Arrow 4 represents the uptake of exogenous sucrose directly into the storage compartment and the double arrow 6 represents an exchange of sucrose between the cell exterior and the storage compartment.[4] Arrows 4, 5, 6 and A may represent different aspects of a single sucrose transport system between the storage compartment and cell exterior. Similarly, arrows 1, 2 and 3 may represent a single transport system between the two cellular compartments. Whether or not the same mechanism for sucrose transport is operative in both cases remains to be established.

The results presented in this paper show that tris(hydroxymethyl)aminomethane (tris) strongly inhibits uptake of sucrose (arrow 4) and increases leakage of stored sucrose (arrow 5) and that the inhibition is reversed by H^+ and either Al^{3+} or certain divalent cations.

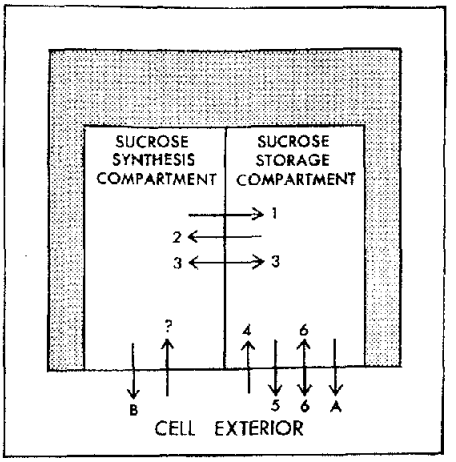

FIG. 1. MOVEMENTS OF SUCROSE IN THE CORN SCUTELLUM CELL.

A single cell is diagramed showing only two compartments while the rest of the cell is represented by the stippled area. The diagram is not meant to signify anything about the size, the number, or the position of the compartments within the cell. The only implications here are that the two compartments are contiguous to one another and to the cell exterior. The arrows indicate direction of sucrose movement. The numbers and letters are explained in the Introduction.

RESULTS

Sucrose Leakage and Storage

We consider sucrose to be stored if it does not leak from the scutellum slices into water, and assume (Fig. 1) that the sucrose is contained in one or more storage compartments in the cell. When freshly prepared, well-washed slices containing, by our definition, only stored sucrose were placed in tris the amount of sucrose in the slices decreased 50·7 μmoles in 3 hr (Table 1). About 30 μmoles of this leaked into the tris solution bathing the slices (Fig. 2) and, presumably, the other 20 μmoles were metabolized. When the slices were incubated with both tris and sucrose, they lost about as much sucrose as when they were incubated in tris alone (Table 1). It appears from these results that tris caused the membrane between the storage compartment and the bathing solution to become leaky (Fig. 1, arrow 5) and perhaps also inhibited the storage of exogenous sucrose (Fig. 1, arrow 4).

When both tris and fructose were present in the bathing solution the greatest leakage of sucrose occurred (Fig. 2) and there also was a small increase in tissue sucrose (Table 1). Undoubtedly, this leakage was comprised of both newly synthesized sucrose and sucrose

TABLE 1. EFFECT OF TRIS BUFFER ON THE SUCROSE CONTENT OF SCUTELLUM SLICES*

Bathing solution		Change in tissue sucrose†
Sugar (0·1 M)	Tris (0·06 M, pH 7·5)	(μmoles/g)
Sucrose	−	(+) 41·0
Sucrose	+	(−) 44·5
Fructose	−	(+) 61·8
Fructose	+	(+) 8·4
Nil	+	(−) 50·7

* The slices (1·0 g fresh wt.) were incubated in the bathing solutions for 3 hr at 30°.

† Slices killed at the beginning of the incubation period contained 65·3 μmoles sucrose per g fresh wt.

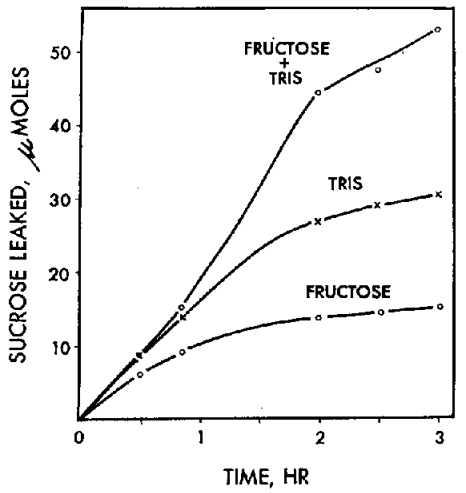

FIG. 2. THE EFFECT OF TRIS AND FRUCTOSE ON THE LEAKAGE OF SUCROSE FROM SCUTELLUM SLICES.

The slices (1·0 g fresh wt.) were incubated at 30° in tris (0·06 M, pH 7·5), in 0·1 M fructose or in tris plus fructose. Portions of these bathing solutions were removed at the times shown and analyzed for sucrose. The results shown here were obtained from the same experiment as those shown in Table 1.

originally occupying the storage compartment of the cell. In this case, however, either the newly synthesized sucrose could have leaked directly from the synthesis compartment to the cell exterior (Fig. 1, arrow B) or could first have been stored and then leaked into the bathing solution as before (Fig. 1, arrow 5). It is important to determine which route the newly synthesized sucrose took in reaching the bathing solution because it would help answer the

question: does tris cause a general leakiness to sucrose in all membranes or is this leakiness due to the action of tris on a sucrose transport system represented by some combination of the arrows 4, 5, 6, A from the storage compartment in Fig. 1?

The results shown in Table 2 indicate that, in the presence of fructose and tris, sucrose leakage originated from the storage compartment. This experiment took advantage of the fact that, as the fructose concentration is increased above 0·1 M, sucrose is synthesized more rapidly than it is stored and sucrose builds up in the synthesis compartment from which it leaks (leakage B) when the slices are placed in water.[2] If tris causes membranes, in general, to become leaky to sucrose then, as the fructose concentration is increased in the presence of tris,

TABLE 2. THE EFFECTS OF TRIS BUFFER AND THE CONCENTRATION OF FRUCTOSE ON THE PRODUCTION, STORAGE AND LEAKAGE OF SUCROSE*

Bathing solution		Sucrose, μmoles				
Fructose molarity	Tris	Net storage†	Leakage† "A" or "5"	= Total storage	Leakage "B"	Total Prod.
0·1	–	54·2	19·4	73·6	1·9	75·5
0·5	–	57·0	16·7	73·7	18·9	92·6
0·8	–	47·2	12·8	60·0	32·8	92·8
0·1	+	12·5	50·0	62·5	8·1	70·6
0·5	+	28·5	38·3	66·8	20·6	87·4
0·8	+	1·4	36·9	38·3	35·8	74·1

* The slices (1·0 g fresh wt.) were incubated in the bathing solutions for 3 hr at 30°. Tris, when used, was present at a concentration of 0·06 M (pH 7·5). At the end of the 3-hr period a portion of the bathing solution was removed for the determination of leakage "A" or arrow "5" sucrose and the slices were quickly washed with 10 ml of water and then 10 ml of water were added. Incubation was continued for an additional 45 min and then a portion of the bathing solution was removed for the determination of leakage B sucrose. The sucrose content of the slices was then determined (see Materials and Methods). Net storage values were calculated by subtracting the amount of sucrose found in slices killed at zero time from those amounts found in the slices after both the 3 hr and 45 min incubations.

† In the presence of only fructose this leakage is leakage "A" but in the presence of fructose and tris this leakage is probably not only "A" but also that represented by arrow 5, Fig. 1.

sucrose should not build up in the synthesis compartment to later leak into water but should leak immediately into the tris-fructose bathing solution. It can be seen (Table 2) that this did not occur and the membrane between the synthesis compartment and the cell exterior remained tight (leakage "B") while the membrane separating the storage compartment from the cell exterior became leaky (leakage "A" or "5"). It should be noted that the addition of tris to the fructose bathing solutions caused a decrease both in the amount of sucrose stored and in the amount produced (Table 2). However, the amount of leakage B sucrose was increased by tris (2–6 μmoles, Table 2), but this increase probably resulted from traces of tris remaining in the slices after the water wash which preceded the measurement of leakage "B" and, therefore, should not be included in the latter (see Fig. 4).

When slices, which had been placed in 1·0 M fructose in order to load the synthesis compartment with sucrose, are placed in solutions of Ca^{2+} or Mn^{2+} instead of water, leakage B

is strongly inhibited.[6] We have suggested that these cations decrease the effective size of the pores in the membrane separating the synthesis compartment from the cell exterior thus preventing sucrose exit.[6] Similar results with Al^{3+} as the inhibitory cation are shown in Fig. 3. Despite the fact that Al^{3+} strongly inhibited the leakage of sucrose across the synthesis

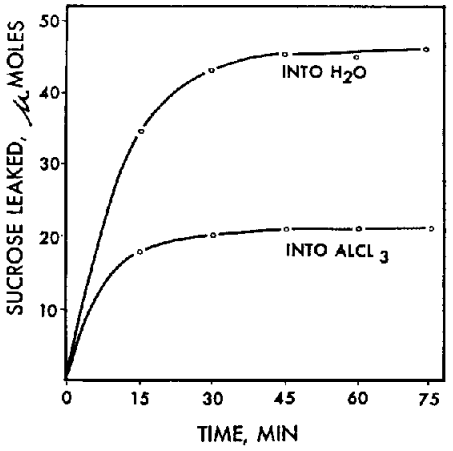

FIG. 3. THE INHIBITION OF SUCROSE LEAKAGE B BY $AlCl_3$.

The slices (1·0 g fresh wt.) were incubated in 1·0 M fructose at 30° for 3 hr. At the end of this period, the bathing solution was removed from the flask by suction, and the slices were washed by the rapid addition and removal of 10 ml of water or $AlCl_3$ (0·02 M). Then 10 ml of water or $AlCl_3$ (0·02 M) were added to the flasks (time zero on the graph). Portions of the bathing solution were removed for sucrose analysis at the times shown.

TABLE 3. EFFECT OF PRIOR TREATMENT IN TRIS ON SUCROSE STORAGE*

Bathing solution		Sucrose stored, μmoles†	
Sugar (0·1 M)	$AlCl_3$ (0·02 M)	H_2O pretreatment	Tris pretreatment
Sucrose	–	54±5 (10)	5±4 (6)
Sucrose	+	62±8 (5)	48±6 (7)
Fructose	–	80±6 (8)	69±4 (4)
Fructose	+	92±3 (4)	77±5 (4)

* The slices (1·0 g fresh wt.) were incubated in water or in tris (0·06 M, pH 7·5) for 1 hr at 30°. The bathing solutions were then removed, the slices were washed with 10 ml of water, and 10 ml of the above bathing solutions were added. The slices were incubated in these solutions for 3 hr at 30°. The sucrose content of the slices was determined following the procedures outlined in Materials and Methods.

† The numbers in both columns are averages followed by the standard deviation and, in brackets, the number of values, each from a separate group of scutellum slices, upon which the average was based.

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

compartment membrane it did not prevent movement of exogenous sucrose into the storage compartment. Indeed, the storage of sucrose in slices bathed in either sucrose or fructose was increased by the presence of Al^{3+} (Table 3, first column). If, in fact, Al^{3+} decreases the leakage of sucrose from the synthesis compartment (Fig. 1, arrow B) by diminution of pore size, it would follow that Al^{3+} would also restrict the entry of sucrose from the bathing medium into the synthesis compartment (Fig. 1, reversal of arrow B). Following this line of reasoning, these results (Table 3) indicate that the storage of exogenous sucrose occurs from the bathing solution directly into the storage compartment. These results, however, do not rule out a sucrose transport system (Fig. 1, arrow ?) for the movement of exogenous sucrose into the synthesis compartment. Al^{3+} did not inhibit the movement of fructose into the synthesis compartment nor did it inhibit the subsequent synthesis and storage of sucrose (Table 3).

The above results show that the membranes separating the two cellular compartments from the cell exterior are different and that tris inhibits the storage of exogenous sucrose and causes the storage compartment membrane to become leaky.

Reversal of Tris Inhibition of Sucrose Storage

When slices were treated by incubation in tris (0·06 M, pH 7·5) for 1 hr at 30° followed by a single water wash, their ability to store exogenous sucrose was decreased approximately 90 per cent (Table 3). In contrast, the synthesis and storage of sucrose when the treated slices were placed in fructose was decreased only about 15 per cent. Furthermore, after tris treatment the slices were no longer leaky (cf. Fig. 4 with Fig. 2). There was some sucrose leakage when the tris-treated slices were placed in water or in fructose, but this was essentially completed in the first hour (Fig. 4). This leakage probably was caused by residual tris which leached from the slices during the first hour and was diluted by the water or fructose solution bathing the slices.

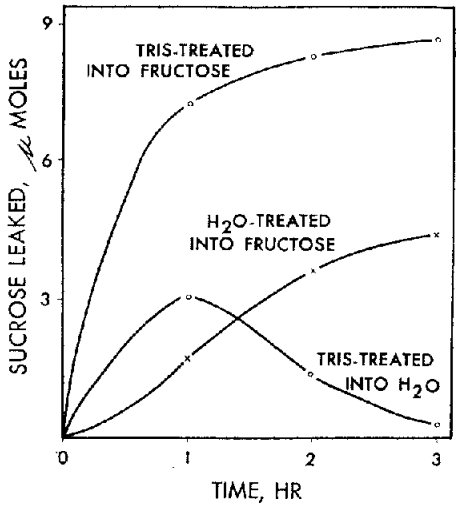

FIG. 4. LEAKAGE OF SUCROSE FROM SLICES AFTER TRIS TREATMENT.

The slices (1·0 g fresh wt.) were placed in tris (0·06 M, pH 7·5) at 30° for 1 hr. At the end of this period, the tris solution was removed by suction, and the slices were washed by the rapid addition and removal of 10 ml of water. Then 10 ml of water or 0·1 M fructose were added to the flasks (time zero on the graph). Portions of the bathing solutions were removed for sucrose analysis at the times shown. To serve as a control, one group of slices was placed in water instead of tris. These slices were washed and 0·1 M fructose was added.

The property of Al^{3+} in reversing tris inhibition of exogenous sucrose storage is shown in Table 3. However, since $AlCl_3$ solutions have a low pH, it was necessary to distinguish

TABLE 4. EFFECT OF H^+ AND Al^{3+} ON SUCROSE STORAGE IN SLICES PRETREATED WITH TRIS BUFFER*

Bathing solution (sugar (0·1 M))	Additions	Sucrose stored, μmoles	Final pH†
Sucrose	Nil	2·6	5·4
	HCl (10^{-3} M)	5·5	4·7
	HCl (10^{-2} M)	25·7	2·2
	$AlCl_3$ (0·02 M)	42·3	2·6
	HCl (10^{-3} M) + $AlCl_3$ (0·02 M)	40·2	2·4
	HCl (10^{-2} M) + $AlCl_3$ (0·02 M)	43·0	1·6
Fructose	Nil	66·0	5·5
	HCl (10^{-3} M)	66·6	5·3
	HCl (10^{-2} M)	58·3	2·2
	$AlCl_3$ (0·02 M)	70·6	2·6
	HCl (10^{-3} M) + $AlCl_3$ (0·02 M)	71·3	2·5
	HCl (10^{-2} M) + $AlCl_3$ (0·02 M)	65·9	1·6

* The slices (1·0 g fresh wt.) were pretreated in tris buffer and then washed and placed in the above bathing solutions for 3 hr at 30° (see footnote, Table 3).

† The figures refer to the pH of the bathing solution at the end of the 3 hr incubation.

TABLE 5. EFFECT OF CATIONS ON SUCROSE STORAGE IN SLICES PRETREATED WITH TRIS BUFFER*

Bathing solution (sucrose (0·1 M) + additions)	Sucrose stored, μmoles†	Final pH
+ Nil	8·0	5·6
+ HCl (10^{-2} M)	25·0	2·1
+ HCl (10^{-2} M) + $AlCl_3$ (0·02 M)	40·3	1·6
+ HCl (10^{-2} M) + $MnCl_2$ (0·02 M)	41·7	1·7
+ HCl (10^{-2} M) + $CoCl_2$ (0·02 M)	33·7	1·8
+ HCl (10^{-2} M) + $MgCl_2$ (0·02 M)	30·1	1·9
+ HCl (10^{-2} M) + $CaCl_2$ (0·02 M)	25·7	1·8

* The slices (1·0 g fresh wt.) were pretreated in tris buffer and then washed and placed in the above bathing solutions for 3 hr at 30° (see footnote, Table 3). The pH of the bathing solution was determined at the end of the 3 hr incubation.

† These figures are averages of the values from two experiments.

between the effect of pH and the effect of Al^{3+} on the reversal of tris inhibition. The results shown in Table 4 demonstrate that both H^+ and Al^{3+} caused an increase in the storage of exogenous sucrose. Al^{3+} had an effect over and above that which could be attributed to H^+

concentration. Al^{3+} was tested in this system only at pH's of 2·6 or below. When fructose was the sugar source, however, the addition of HCl caused a small decrease while $AlCl_3$ caused a small increase in sucrose storage (Tables 3 and 4). The effects of Mn^{2+}, Co^{2+}, Mg^{2+} and Ca^{2+} on the storage of exogenous sucrose in tris-treated slices are shown in Table 5. Since solutions of the chloride salts of these cations had a pH of 4 or above it was necessary to add HCl when comparing them with $AlCl_3$. Mn^{2+} was as effective as Al^{3+} in reversing the inhibition, Co^{2+} and Mg^{2+} caused only half as much increase in sucrose storage as did Al^{3+} or Mn^{2+}, and Ca^{2+} had no effect on sucrose storage. In the absence of HCl the divalent cations either had no effect on or increased the inhibition of exogenous sucrose storage.

DISCUSSION

The results of this and previous papers[1, 2, 4, 6] clearly show that the properties of the membrane separating the sucrose synthesis compartment from the cell exterior (the synthesis membrane) are different from those of the membrane separating the storage compartment from the cell exterior (the storage membrane). Free diffusion of sucrose does not take place through the storage membrane. However, this membrane contains a transport system for sucrose uptake,[4] it exhibits a special type of leakiness towards sucrose which is perhaps just one aspect of the transport system (leakage A)[2] and it becomes leaky to sucrose in the presence of tris (Fig. 2) and other buffers.[4] In contrast, the synthesis membrane is leaky towards sucrose (leakage B) but becomes "tight" in the presence of high concentrations of hexose or polyhydric alcohols[2] and in the presence of divalent and trivalent cations (Fig. 3 and Ref. 6). Furthermore, the "tightness" of the synthesis membrane to sucrose caused by high fructose concentrations is not lessened by the addition of tris (Table 2, the leakage B data). We conclude that the synthesis membrane contains pores large enough for the passage of sucrose while the storage membrane does not. Furthermore, since the hexose space (the volume of tissue water necessary to contain the hexose of the tissue at the concentration of the bathing solution[7]) makes up only about 12 per cent of the tissue water of the corn scutellum,[1] and since the sucrose synthesis compartment must be included as part (or all) of the hexose space, we conclude that the sucrose storage compartment is outside the hexose space. If this is correct then the synthesis membrane allows the free diffusion of hexose while the storage membrane does not.

The membrane separating the synthesis and storage compartments (the intercompartmental membrane) is presumed to be very much like the storage membrane in regard to sucrose and hexose movement. This membrane contains a sucrose transport system[2, 6] and must not allow free diffusion of sucrose or hexose. However, while the storage membrane is at the cell exterior and is markedly influenced by the exterior environment, the membrane separating the two compartments is removed from the cell exterior and is much less influenced by exterior conditions (this paper and Ref. 4).

The mechanism of the tris effect on the storage membrane causing the membrane to become leaky and inhibiting the storage of exogenous sucrose is unknown. Citrate–phosphate buffers (0·06 M) in the pH range 5·0–7·3 also increased the leakiness of the storage membrane and inhibited sucrose storage.[4] These effects of citrate–phosphate buffers became more pronounced as the pH increased but even at pH 7·3 they were not as great as those produced by tris (pH 7·5). Tris, as a large monovalent cation, may disrupt protein–phospholipid bonds or protein–protein bonds in the membrane. The pH may influence the degree of

[7] H. E. MORGAN, M. J. HENDERSON, D. M. REGEN and C. R. PARK, *J. Biol. Chem.* **236**, 253 (1961).

aggregation of membrane protein[8] and also the net charge of intact membrane.[9] Possibly, then, pH changes may alter the architecture and properties of membranes. Since the effects of tris were reversible a major disruption of membrane structure must not have occurred.

In many studies using a variety of plant and animal tissues, divalent cations (especially Ca^{2+}) have been shown to make membranes less permeable towards both ions and neutral compounds.[6, 10–12] Tissues made leaky by incubation in EDTA become "tight" again upon the addition of Ca^{2+}. It has been proposed that divalent cations bind within negatively charged pores which transverse the membrane, and that this binding results in a decrease in the pore diameter.[12, 13] In the corn scutellum the leakage of sucrose from the synthesis compartment (leakage B) is strongly inhibited by Ca^{2+}, Mn^{2+}, Zn^{2+}, Co^{2+} and Al^{3+} (unpublished data, Fig. 3 and Ref. 6). Leakage B also is inhibited by high concentrations of hexose or polyhydric alcohols.[2] These results suggest that leakage B sucrose moves through pores in the synthesis membrane whose diameter is controlled to some extent by the cation content and water potential of the surrounding solution. In contrast, the tris-induced leakiness in respect to sucrose exhibited by the storage membrane disappeared upon removal of the tris, and the addition of cations was required not to tighten a leaky membrane but rather to reinitiate the storage of exogenous sucrose. We conclude that the inhibition of sucrose storage in the presence of tris primarily is due to the action of tris on the storage mechanism.

It is notable that a low pH is required before the cations are effective in reversing the tris-induced inhibition of sucrose storage, and it is surprising that Ca^{2+} is inactive. However, the cations were tested at only two pH values (in 10^{-2} M HCl or at the pH of 0·02 M solutions of their chloride salts), and activity vs. pH curves were not determined. These results indicate that the divalent cations or Al^{3+} undergo a pH-dependent interaction with the lipids and/or proteins of the membrane re-establishing a structure altered by tris.

EXPERIMENTAL

Plant Materials

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 in the Results section of this paper, certain methods remained the same throughout these investigations. Each group of slices (1 g fresh 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 A and leakage B and the preparation of the ethanolic extracts of the tissue slices have been described previously.[6]

[8] J. M. Steim and S. Fleischer, *Proc. Nat. Acad. Sci., U.S.* **58**, 1292 (1967).
[9] D. F. H. Wallach, V. B. Kamat and M. H. Gail, *J. Cell. Biol.* **30**, 601 (1966).
[10] B. D. Foote and J. B. Hanson, *Plant Physiol.* **39**, 450 (1964).
[11] W. J. V. Osterhout, *Injury, Recovery and Death in Relation to Conductivity and Permeability*, J. B. Lippincott, Philadelphia and London (1922).
[12] G. Whittembury, N. Sugino and A. K. Solomon, *Nature* **187**, 699 (1960).
[13] A. K. Solomon, in *Membrane Transport and Metabolism* (edited by A. Kleinzeller and A. Kotyk), p. 329, Academic Press, New York (1961).

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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