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THE EFFECT OF D-MANNOSE ON SUCROSE STORAGE IN THE CORN SCUTELLUM: EVIDENCE FOR TWO SUCROSE TRANSPORT MECHANISMS*

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Abstract—Evidence is presented that tissue slices of the corn scutellum contain two distinct mechanisms for the storage of sucrose. The first mechanism is responsible for the storage of exogenously supplied sucrose and is strongly inhibited by the presence of D-mannose in the sucrose-containing bathing medium. The second mechanism is responsible for the transport of sucrose (newly synthesized from hexose) from the synthesis compartment of the cell into storage. This mechanism is not inhibited by mannose. Although the intercompartmental storage of newly synthesized sucrose is unaffected by the presence of mannose, the synthesis of the disaccharide from exogenously supplied fructose is strongly inhibited when mannose is present in the bathing medium. In addition, treatment of tissue slices with mannose resulted in a drastic reduction in the ATP level of the tissue. This reduction in ATP was not accompanied by an equivalent increase in ADP or AMP. It is suggested that the effect of mannose in inhibiting the storage of exogenous sucrose and in inhibiting the synthesis of sucrose from exogenous fructose arises from ATP deficiency. This deficiency is presumably the result of mannose and mannose-6-phosphate inhibitions of glycolysis at the level of hexokinase and phosphoglucose isomerase, respectively, and/or the trapping of inorganic phosphate in a pool of non-metabolizable mannose-6-phosphate. Possible mechanisms for sucrose storage are discussed.

INTRODUCTION

INVESTIGATIONS conducted in this laboratory allow us to visualize to some extent the structure and function of the parenchymatous cell of the corn scutellum in regard to sugar uptake, sugar interconversions and sugar storage.[1–5] The presentation of the data of this paper will be facilitated by the definition of certain terms used here and in previous reports and also by the diagramatic portrayal of the scutellum cell (Fig. 1).

The *sucrose storage compartment* is that volume of the cell from which sucrose will not leak into water, and it is also the origin of leakage A sucrose.[2] The *sucrose synthesis compartment* is that volume of the cell within which sucrose or a sucrose derivative is produced from hexose. It is believed to be the "hexose space"[1] and also to contain the glycolytic sequence.[5] This compartment is also referred to as the "B compartment" because it is the origin of leakage B sucrose.[2] The *storage membrane* is that membrane separating the sucrose storage compartment and the cell exterior. The *synthesis membrane* is that membrane separating the sucrose synthesis compartment and the cell exterior; this membrane is freely permeable to hexoses,[1] but restricts the entry of sucrose (unpublished). The *intercompartmental*

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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.* **6**, 1085 (1967).
[4] T. E. HUMPHREYS and L. A. GARRARD, *Phytochem.* **7**, 701 (1968).
[5] L. A. GARRARD and T. E. HUMPHREYS, *Phytochem.* **7**, 1949 (1968).

membrane separates the sucrose synthesis and storage compartments. *Leakage A* is stored sucrose which leaks from the tissue slices during their incubation in fructose; this leakage is accompanied by an exchange reaction between the fructose of the bathing solution and the fructose moiety of the stored sucrose and represents a loss of stored sucrose from the tissue.[2] *Leakage B* is newly synthesized sucrose which leaks from the synthesis compartment into water following experimental conditions which allow accumulation of sucrose or a derivative of sucrose in the synthesis compartment.[2,3] *Stored sucrose* is that sucrose contained in the tissue slices which will not leak from the tissue into water. The sucrose content of freshly prepared tissue slices is considered to be solely stored sucrose.[2–4]

The highly schematic portrayal of a single parenchymatous cell of the corn scutellum (Fig. 1) provides a simplistic view of the above-mentioned terms. Results of previous studies have suggested that hexoses (fructose or glucose) freely diffuse into the sucrose synthesis

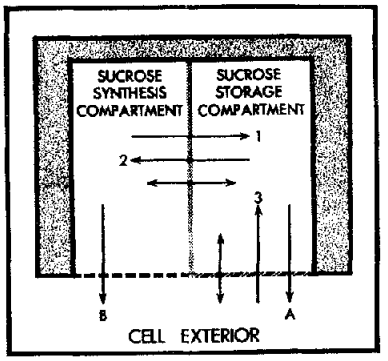

FIG. 1. MOVEMENTS OF SUCROSE IN THE CORN SCUTELLUM CELL.

A single cell is shown with 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 the direction of sucrose movement while the numbers and letters are explained in the Introduction. The various membranes have been portrayed as follows: ⁘⁘⁘⁘⁘⁘⁘⁘⁘ is the intercompartmental membrane, ▬ ▬ ▬ is the synthesis membrane, and ▰▰▰▰ is the storage membrane. This portrayal is not meant to signify anything about the structure of the membranes.

compartment and rapidly establish an equilibrium with the hexose concentration of the bathing solution (within 30 min).[1] Hexoses do not accumulate in the tissue but rather are utilized for the production of sucrose.[1,6] Sucrose storage (arrow 1, Fig. 1) occurs across the intercompartmental membrane and is not obligatorily coupled to sucrose synthesis.[2] At high fructose concentrations in the bathing solution (above 0·1–0·2 M), the storage of newly synthesized sucrose is inhibited and sucrose or a derivative of sucrose accumulates in the synthesis compartment. This accumulated sugar readily leaks into the bathing medium when the fructose is diluted or replaced by water (arrow B, Fig. 1).[2] This leakage B occurs across the synthesis membrane into the bathing solution. If slices containing accumulated sucrose or sucrose derivative in the synthesis compartment are placed in solutions of $CaCl_2$, leakage B is inhibited and the sucrose or derivative of sucrose is transported from the synthesis compartment to the storage compartment.[3] Leakage A (arrow A, Fig. 1) represents the loss of stored sucrose during incubation of tissue slices in fructose. This leakage occurs across

[6] T. E. HUMPHREYS and L. A. GARRARD, *Phytochem.* **6**, 647 (1964).

the storage membrane and appears to be relatively independent of external fructose concentration over a wide range.[2] However, the amount of leakage A is greatly reduced by incubation of tissue slices in water for 60–90 min prior to their treatment in fructose.[4] In the absence of exogenous sugar, the tissue slices utilize 5–6 μmoles of sucrose/g/hr. This metabolic use of stored sucrose is denoted by arrow 2, Fig. 1. Arrow 2 is directed into the synthesis compartment because this volume of the cell is thought to contain the glycolytic sequence.[5] In addition to the storage of sucrose or a sucrose derivative synthesized from exogenously supplied hexose, the scutellum cell has a transport mechanism for the storage of exogenous sucrose. This storage of sucrose takes place directly from the bathing solution across the storage membrane into the storage compartment (arrow 3, Fig. 1).[4] The bidirectional arrows shown in Fig. 1 represent mechanisms for sucrose exchange and do not denote mechanisms which have roles in net sucrose movement.[2,4] However, these exchange phenomena may represent some aspect of mechanisms capable of sucrose transport and storage.

The present investigations are concerned with the effect of D-mannose on the leakage and storage of sucrose by the corn scutellum cell. Mannose has been found to strongly inhibit the uptake of glucose[6] and fructose (unpublished) from solutions bathing the tissue slices; however, mannose does not inhibit the entry of glucose and presumably fructose into the sucrose synthesis compartment.[1] In addition, mannose appears to readily enter the sucrose synthesis compartment (hexose space) and come to rapid equilibrium with mannose concentrations of the bathing solution.[1] It is apparent then that the effect of mannose must be on the intracellular utilization of glucose and fructose (i.e. the synthesis of sucrose) and thereby may serve as a tool in the further study of sucrose synthesis and storage.

RESULTS

The Effect of Mannose on the Synthesis and Storage of Sucrose

Experiments were conducted to determine the effect of mannose on the synthesis and storage of sucrose from an exogenous supply of fructose and on the storage of exogenous sucrose. Tissue slices were first incubated in water for 90 min (to reduce the amount of leakage A[4]) after which they were placed in either 0·1 M fructose or 0·1 M fructose plus various concentrations of mannose. Similarly, groups of tissue slices were placed in either 0·3 M sucrose or 0·3 M sucrose plus mannose. These concentrations of fructose or sucrose were used because they gave nearly maximum rates of sucrose storage. Incubation in the sugar solutions was continued for 2 hr after which the slices were placed in water and allowed to leak unstored sucrose for a period of 30 min (maximum leakage occurred during this period). The sucrose remaining in the tissue slices following their incubation in water was considered to be solely stored sucrose. Thus, with sucrose incubations, sucrose storage during the 2-hr incubation period was equivalent to the increase in the tissue content of the disaccharide. In the case of fructose incubations, sucrose synthesis was equivalent to the sum of leakage A, leakage B and the increase in the tissue content of sucrose while total sucrose stored during the incubation period was equivalent in amount to the sum of leakage A and the increase in the tissue content of sucrose (or also, the amount of newly synthesized sucrose minus the amount of leakage B).

As can be seen in Fig. 2, the presence of mannose in the bathing medium strongly inhibited the synthesis of sucrose from exogenously supplied fructose (curve 1), and that approximately 50 per cent inhibition resulted from the use of 0·005 M mannose. In addition, the presence of mannose strongly inhibited the storage of exogenously supplied sucrose (curve 2) although

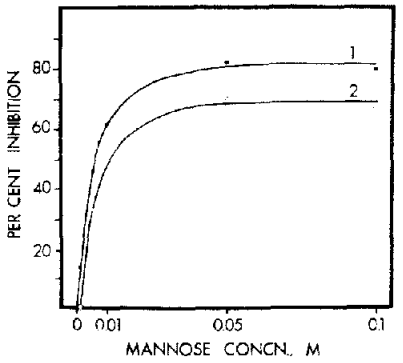

FIG. 2. THE EFFECT OF THE PRESENCE OF MANNOSE IN THE BATHING MEDIUM ON THE SYNTHESIS OF SUCROSE FROM EXOGENOUS FRUCTOSE AND THE STORAGE OF EXOGENOUS SUCROSE.

The slices (1·0 g fresh wt.) were incubated in distilled water for 90 min at 30° after which the bathing solution was removed by suction and 10 ml of either 0·3 M sucrose or 0·1 M fructose each containing various concentrations of mannose were added to the flasks. The slices were then incubated for 120 min at 30° in the various sugar solutions after which the bathing media were removed (in the case of fructose incubations, a sample was taken of the bathing medium for leakage A determination), the slices washed briefly in distilled water, and 10 ml of water added to the flasks. Incubation in water was continued for 30 min in order to remove leakable sucrose. In the case of fructose incubations, a portion of this final bathing solution was collected for the determination of leakage B sucrose. The tissue slices were then extracted with hot ethanol and the sucrose contents of the tissue determined. The percentages of inhibition given are based on control flasks which contained no mannose. Curve 1 is for sucrose synthesis from exogenous fructose while Curve 2 is for sucrose storage from exogenous sucrose.

in this case a concentration of 0·01 M mannose was required to produce 50 per cent inhibition of storage. It is apparent from these data that mannose enters the synthesis compartment of the scutellum cell and inhibits sucrose synthesis from exogenous fructose and that mannose also inhibits the storage of exogenous sucrose, a process which does not require inversion and resynthesis of the disaccharide and which is believed to take place directly from the bathing medium to the storage compartment across the storage membrane.[4] The question remains, however, whether or not mannose inhibits the storage of newly synthesized sucrose (i.e. the transport of sucrose from the synthesis compartment into the storage compartment across the intercompartmental membrane). The data given in Table 1 show that nearly all of the

TABLE 1. THE EFFECT OF MANNOSE IN THE BATHING MEDIUM DURING INCUBATION IN FRUCTOSE ON THE STORAGE OF NEWLY SYNTHESIZED SUCROSE*

Bathing medium during 2-hr incubation period	Sucrose (μmoles/g fresh wt.)							
	Leakage				Increase in tissue		Synthesized	Stored
	A		B					
Fructose, 0·1 M	5·2	+	1·3	+	67·2	=	73·7	72·4
Fructose + 0·001 M mannose	5·2	+	1·4	+	56·1	=	62·7	61·3
Fructose + 0·005 M mannose	4·7	+	1·1	+	33·9	=	39·7	38·6
Fructose + 0·01 M mannose	4·1	+	1·0	+	22·8	=	27·9	26·9
Fructose + 0·05 M mannose	5·0	+	1·5	+	6·7	=	13·2	11·7
Fructose + 0·1 M mannose	5·1	+	1·9	+	7·8	=	14·8	12·9

* For experimental procedures, see caption, Fig. 1.

newly synthesized sucrose produced in tissue slices bathed in 0·1 M fructose was stored by the scutellum cell (leakage B was small). In addition, the presence of mannose in the fructose bathing medium appeared to have no effect on the storage of newly synthesized sucrose over a wide range of mannose concentrations (leakage B remained small). It is also interesting to note that the presence of mannose in the bathing medium had little if any effect on the loss of stored sucrose across the storage membrane by the leakage A mechanism. Clearly, these results suggest either that the storage membrane and intercompartmental membrane have decidedly different mechanisms for sucrose transport (the former inhibited by mannose, the latter not inhibited by mannose) or that a derivative of sucrose is produced in the synthesis compartment which may be stored in the presence of mannose.

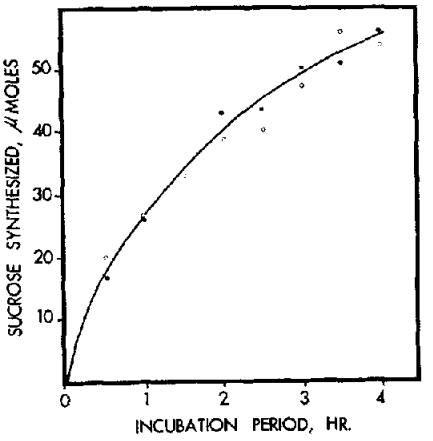

FIG. 3. THE TIME COURSE OF SUCROSE SYNTHESIS FROM AN EXOGENOUS SUPPLY OF FRUCTOSE CONTAINING AN INHIBITORY LEVEL OF MANNOSE.

The slices (1·0 g fresh wt.) were incubated in distilled water for 90 min at 30° after which the water was removed by suction and 10 ml of a solution containing 0·1 M fructose plus 0·05 M mannose were added. The slices were incubated at 30° for the various time periods given above after which a sample of the bathing medium was collected for the determination of leakage A sucrose and the tissue slices extracted with hot ethanol for the determination of tissue sucrose (stored sucrose + leakage B sucrose). In addition, a duplicate flask was incubated in fructose plus mannose for the entire 4-hr period after which the slices were washed briefly in distilled water and allowed to leak sucrose into water. This leaked sucrose was a measure of the unstored, newly synthesized sucrose (leakage B sucrose) which amounted to only 1·2 μmoles.

The time course (4 hr) of sucrose synthesis from exogenous 0·1 M fructose in the presence of 0·005 M mannose is shown in Fig. 3. The concentration of mannose used was that which gave approximately 50 per cent inhibition of sucrose synthesis (Fig. 2). Inasmuch as virtually all the sucrose synthesized under these experimental conditions was stored by the cells (leakage B was 1·2 μmoles after 4 hr), the sucrose synthesis curve would very closely resemble a sucrose storage curve for these data. It may be seen in Fig. 3 that the rate of sucrose synthesis declined with time during incubation of the tissue slices in 0·1 M fructose plus 0·005 M mannose; however, under these conditions, the decline in sucrose storage (transport from the synthesis compartment to the storage compartment) must be attributed to a decrease in the amount of sucrose or sucrose derivative available for storage rather than any direct effect of mannose on the intercompartmental sucrose storage mechanism.

The time course (4 hr) of sucrose storage from exogenous 0·3 M sucrose in the presence

of 0·01 M mannose is shown in Fig. 4. Again, the concentration of mannose used was that which gave approximately 50 per cent inhibition of sucrose storage during a 2-hr incubation period (Fig. 2). There was a decrease in the rate of sucrose storage during the first 30 min of incubation followed by a constant rate thereafter. It is probable that the initial faster rate of sucrose storage occurred before mannose had a chance to act either directly or indirectly on the storage mechanism. In any event, these data reinforce previous evidence[4] for a mechanism for the storage of exogenous sucrose without inversion and resynthesis of the disaccharide. If inversion and resynthesis were necessary for sucrose storage, then the curve presented in Fig. 4 should resemble that obtained when fructose was provided as the sugar source for sucrose synthesis (Fig. 3). It is also unlikely that sucrose is being stored by entering the synthesis compartment and utilizing the intercompartmental storage mechanism because this

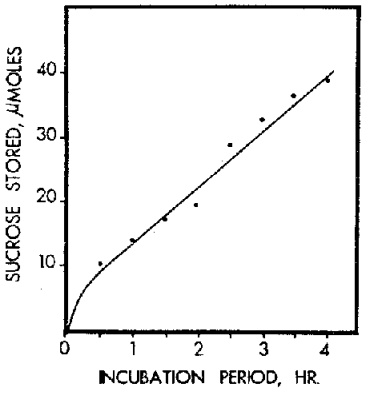

FIG. 4. THE TIME COURSE OF SUCROSE STORAGE FROM AN EXOGENOUS SUPPLY OF SUCROSE CONTAINING AN INHIBITORY LEVEL OF MANNOSE.

The slices (1·0 g fresh wt.) were incubated in distilled water for 90 min at 30° after which the water was removed from the flask by suction and 10 ml of a solution containing 0·3 M sucrose plus 0·01 M mannose were added. The slices were incubated for the various periods given above after which the sugar-containing bathing medium was removed, the slices washed briefly with distilled water, and the tissue placed in 10 ml of distilled water. The slices were incubated for an additional 30-min period to remove leakable sugars and then were extracted with hot ethanol. All the sucrose found in the tissue at this time was considered to be stored sucrose.

mechanism has been shown to be unaffected by the presence of mannose while the storage of exogenous sucrose is strongly inhibited by mannose.

The above data on fructose incubations may be interpreted as showing a very tight coupling between sucrose synthesis and sucrose movement into storage, perhaps even a combined synthesis-storage pathway involving simultaneous production within and movement of sucrose across the intercompartmental membrane. Thus, fructose may be taken up at the surface of the intercompartmental membrane and used for sucrose synthesis within the membrane with sucrose being released within the storage compartment. If this were true, the effect of mannose on intercompartmental sucrose storage would merely be a reflection of its effect on sucrose synthesis.

In order to clearly demonstrate the synthesis of sucrose or a sucrose derivative within the synthesis compartment itself and to show the effect of mannose on the storage of this product, additional studies were made under experimental conditions which allowed an accumulation of unstored sucrose or sucrose derivative within the synthesis compartment.

Tissue slices were incubated in 1·0 M fructose for 3 hr during which more than 40 μmoles of sucrose or sucrose derivative accumulated in the synthesis compartment (high concentrations of exogenous fructose inhibit both leakage B and sucrose storage from the synthesis compartment but do not inhibit the synthesis of sucrose or sucrose derivative[2]). This accumulated sucrose leaked readily into both water and 0·1 M mannose. Groups of slices which had been loaded with unstored sucrose in the above manner were placed for 1 hr in either 0·9 M mannitol or 0·9 M mannitol plus 0·1 M mannose. The high mannitol concentration prevented both the leakage and storage of the newly synthesized sucrose,[2] and the 1-hr period was sufficient for the mannose to enter the synthesis compartment during a period when it contained an appreciable quantity of unstored sucrose or sucrose derivative. Following a 60-min incubation in mannitol or mannitol plus mannose, the slices were incubated for 60 min in 0·1 M $CaCl_2$ or 0·1 M $CaCl_2$ plus 0·1 M mannose after which they were placed in water for an additional 60-min period to allow leakage of unstored sucrose (leakage B). During incubation of tissue slices in $CaCl_2$, sucrose leakage from the synthesis compartment is inhibited but the storage of newly synthesized sucrose is not.[3] Thus, using this type of experiment, it was possible to study the effect of mannose on the storage of sucrose via the intercompartmental storage mechanism under conditions which provided: (1) a large amount of sucrose or sucrose derivative in the synthesis compartment available for storage, (2) adequate time for mannose to enter the synthesis compartment prior to the storage or leakage of sucrose, and (3) an opportunity for intercompartmental sucrose storage to take place both in the presence and absence of mannose during a period of Ca^{2+}-inhibited sucrose leakage to the cell exterior (leakage B).

The results of such an experiment are shown in Table 2. As can be seen, the leakages of unstored sucrose of the synthesis compartment during incubation in mannitol (A) and mannitol plus mannose (B) were small and approximately the same after the 60-min incubation period (8·1 and 6·7 μmoles/g fresh wt., respectively). When, however, the slices from each treatment were subsequently placed in water for 60 min, the leakage of sucrose from slices treated with mannitol plus mannose was greater than the leakage from slices treated with mannitol alone (55·9 and 41·8 μmoles/g fresh wt., respectively). This difference in leakage between the two treatments was not due to a difference in sucrose production but rather a difference in sucrose storage. Slices which leaked the most sucrose had less stored sucrose (compare A-1 with B-1, Table 2). It is possible here that some of the sucrose that leaked into water after mannitol incubation was taken up and stored by the mechanism for storage of exogenous sucrose (Fig. 1, arrow 3), but in the case of mannitol plus mannose treatment the storage of leaked sucrose was inhibited. The presence of Ca^{2+} in the bathing medium following the incubation of tissue slices in mannitol alone strongly inhibited the leakage of sucrose from the synthesis compartment to the cell exterior (compare A-1 with A-2, Table 2), and during the period of Ca^{2+}-inhibited sucrose leakage, most of the sucrose of the synthesis compartment was stored. That the sucrose was stored is shown by the fact that leakage into water following incubation in Ca^{2+} was small while the tissue level of sucrose increased during the incubation period (Table 2, A-2). The presence of Ca^{2+} in the bathing medium following incubation in mannitol plus mannose also inhibited the leakage of unstored sucrose of the synthesis compartment to the cell exterior (compare B-1 with B-2, Table 2); however, the degree of inhibition of sucrose leakage was less than observed when the tissue slices had been incubated in mannitol alone (compare A-1 and A-2 with B-1 and B-2, Table 2). Again, the sucrose not leaked to the cell exterior during incubation in Ca^{2+} was stored (see B-2, Table 2, leakage into water was small and tissue sucrose increased during incubation in Ca^{2+}). It is

most significant that the addition of mannose to the bathing medium during incubation in Ca^{2+} gave essentially the same results for leakage and storage of sucrose as did Ca^{2+} alone (compare B-2 with B-3, Table 2). While the leakages of the two treatments were of the same magnitude, somewhat more tissue (stored) sucrose was found when mannose was present during the period of Ca^{2+}-inhibited sucrose leakage. This difference (9·2 μmoles/g fresh wt.) was probably the result of the restricted metabolic utilization of sucrose caused by the presence

TABLE 2. THE EFFECT OF MANNOSE ON THE STORAGE OF ACCUMULATED SUCROSE OR SUCROSE DERIVATIVE OF THE SYNTHESIS COMPARTMENT*

Treatment	Sucrose (μmoles from 1·0 g) Leakage	Tissue
A. Mannitol, 0·9 M (60 min)	8·1	
Followed by:		
1. Water (60 min)	41·8	84·7
2. $CaCl_2$, 0·1 M (60 min)	9·8	
and then by water (60 min)	5·1	113.1
B. Mannitol, 0·9 M + mannose, 0·1 M (60 min)	6·7	
Followed by:		
1. Water (60 min)	55·9	74·7
2. $CaCl_2$, 0·1 M (60 min)	27·3	
and then by water (60 min)	7·8	90·8
3. $CaCl_2$, 0·1 M + mannose, 0·1 M (60 min)	23·6	
and then by water (60 min)	9·8	100·0

* The tissue slices (1 g fresh wt.) were incubated at 30° for 3 hr in 1·0 M fructose in order to load the synthesis compartment with sucrose or a derivative of sucrose. This sucrose or derivative of sucrose is accumulated in the synthesis compartment, but by our definition, does not represent stored sucrose. Following the initial incubation in fructose, the bathing solutions were removed from the flasks by suction and the slices washed once by the rapid addition and removal of 10 ml of either 0·9 M mannitol or 0·9 M mannitol + 0·1 M mannose. Immediately after washing, 10-ml portions of either mannitol or mannitol + mannose were added to the flasks and the incubation continued as outlined in the above sequences. During each change of bathing solution, the slices were washed briefly, as before, with 10 ml of a solution identical to that into which they were subsequently placed. After each 60-min incubation period, samples were taken from the bathing media to determine sucrose leakage, and at the end of each sequence for a particular treatment, the tissue was killed and extracted with hot ethanol. Tissue sucrose (in this case stored sucrose) was determined by the analysis of these extracts.

of mannose or derivative of mannose. The results of this experiment show that sucrose or a derivative of sucrose which has been allowed to accumulate in the synthesis compartment is readily stored by the intercompartmental storage mechanism in the presence of mannose. Thus, if intercompartmental storage (Fig. 1, arrow 1) involves movement of free sucrose from the synthesis compartment into storage, the mechanism for this storage differs from the mechanism for storage of exogenously supplied sucrose (Fig. 1, arrow 3) the former being insensitive to mannose while the latter is strongly inhibited by mannose. It was therefore necessary to identify the accumulated product in the synthesis compartment.

The Occurrence of Free Sucrose in the Synthesis Compartment

Experiments were conducted to determine whether free sucrose or a derivative of sucrose, perhaps sucrose phosphate as suggested by Hatch[7] for sugarcane, was the product of disaccharide synthesis that accumulated in the synthesis compartment of the cells during periods of incubation in high concentrations of exogenous fructose. A previous report[5] provided evidence that free sucrose was synthesized in the scutellum cell at the expense of "glycolytic ATP" and that free sucrose might well be the form stored across the intercompartmental membrane. However, since this evidence was of an indirect nature, the possibility still remains that a derivative of sucrose is produced in the synthesis compartment of the cell and is converted to free sucrose both during storage and during leakage to the cell exterior (leakage B).

Tissue slices were incubated for 3 hr at 30° in 1·0 M fructose in order to load the synthesis compartment with product. Following this incubation and without washing, the tissue slices were killed and extracted either by the addition of boiling 80% ethanol or by grinding the slices in cold 10% TCA. In each case, the extracts were concentrated *in vacuo* and samples equivalent to 25 mg of fresh tissue were chromatographed on paper in ethyl acetate–pyridine–water (8:2:1 v/v). This solvent separates sucrose, glucose and fructose while leaving sugar phosphates at the origin of the chromatogram. Sugars were located on the chromatograms by spraying with aniline-diphenylamine and were identified both by the colors of the spots and a comparison of their positions on the paper relative to known sugar markers. Visual examination of the chromatograms revealed only three free sugars. Fructose occurred in the greatest amounts in the extracts because the slices had not been washed to remove the adhering fructose solutions. Large amounts of sucrose were also detected while glucose was found in very much smaller quantities. A faint coloration of the origins of the chromatograms indicated the presence of trace amounts of sugar phosphates in the extracts but the quantities were much too small to indicate any substantial amount of the synthesis compartment product occurring as sucrose phosphate. No difference between the sugar contents of the ethanolic extract and the TCA extract could be detected by paper chromatography.

Additional evidence that free sucrose is synthesized and accumulated in the synthesis compartment of the cell during the incubation of tissue slices in high concentrations of fructose is given in Table 3. The slices were incubated for 3 hr at 30° in 0·5 M fructose (A) and 1·0 M fructose (B) after which the slices were briefly washed and then were killed and extracted with boiling ethanol. The ethanolic extracts were assayed for sucrose. In this case, the tissue slices still contained the synthesis compartment product at the time of extraction (no leakage or storage had occurred). The sucrose contents of the tissue were determined enzymatically using glucose oxidase and yeast invertase, a method which will not measure sucrose phosphate.[8] As can be seen in Table 3, the same amount of sucrose (130·0 μmoles/g fresh wt.) was found in the tissue following the 3-hr incubation period at both levels of exogenous fructose (compare A-1 and B-1, Table 3). This was an increase of approximately 50 μmoles/g fresh wt. (compare A-1 and B-1 with zero time, Table 3). Duplicate groups of slices incubated simultaneously with those above at the same two concentrations of exogenous fructose were quickly washed and placed in water for a 45-min period of incubation to allow sucrose leakage to occur (A-2 and B-2, Table 3). Following this period of leakage, tissue (stored) sucrose and leakage B sucrose were determined. A comparison of the amounts of leakage B and tissue sucrose for the two treatments (A-2 and B-2) show that the higher fructose concentration was most effective in inhibiting sucrose storage (thus, increasing the

[7] M. D. HATCH, *Biochem. J.* **93**, 521 (1964).
[8] L. F. LELOIR and C. E. CARDINI, *J. Biol. Chem.* **214**, 157 (1955).

amount of leakable sucrose, leakage B). Furthermore, the sucrose contents of the slices immediately after fructose incubation were equivalent to the sums of the leaked sucrose and tissue sucrose after 45 min in water. These results clearly show that the sucrose of the synthesis compartment is extractable in ethanol in a form which can be acted upon by yeast invertase. Based upon the above data, we suggest that free sucrose is the product of the synthesis compartment and that free sucrose is the form of the disaccharide accumulated in the synthesis compartment during incubation in high concentrations of fructose.

TABLE 3. THE ACCUMULATION OF FREE SUCROSE IN THE SYNTHESIS COMPARTMENT*

Treatment		Sucrose (μmoles/g fresh wt.)
None (zero time)		80·8
A. 3 hr in 0·5 M fructose:		
1. Tissue level immediately after treatment		130·0
2. Tissue level after leakage into water		110·3
Leakage into water (leakage B)		20·1
	TOTAL	130·4
B. 3 hr in 1·0 M fructose:		
1. Tissue level immediately after treatment		130·0
2. Tissue level after leakage into water		94·7
Leakage into water (leakage B)		38·9
	TOTAL	133·6

* The slices (1·0 g fresh wt.) were incubated for 3 hr at 30° in either 0·5 M or 1·0 M fructose after which they were briefly washed with distilled water and quickly extracted with hot 80% ethanol. These alcoholic extracts were analyzed for total tissue sucrose (stored sucrose + leakable sucrose). Duplicate flasks were prepared and handled in the same manner as above with the exception that the slices were placed in water for a 45-min period of sucrose leakage (leakage B) immediately after their incubation in fructose. Following the period of incubation in water, the slices were killed and extracted with hot ethanol. These extracts were analyzed for tissue sucrose. Tissue sucrose after leakage had taken place was considered to be stored sucrose.

Metabolic Intermediates in Response to Mannose Treatment

During a previous study,[6] it was found that incubation of scutellum slices in a solution that was equimolar (*ca.* 0·05 M) in respect to both glucose and mannose was accompanied by a marked decrease in the rate of glucose uptake, decrease in the tissue levels of glucose-6-phosphate (G-6-P) and fructose-6-phosphate (F-6-P) and the appearance of large amounts of mannose-6-phosphate (M-6-P) in the tissue. In addition, evidence was presented that both free mannose and M-6-P contributed to the inhibition of glucose uptake. During the present study, additional experiments were conducted to determine the effect of treatment of scutellum slices with mannose on the tissue levels of certain metabolic intermediates.

Tissue slices were incubated for 1 hr in 0·05 M mannose after which they were extracted with cold 10% TCA and the extracts analyzed for G-6-P, F-6-P and free adenine nucleotides. The results are shown in Table 4. Again, it was apparent that treatment with mannose caused a drastic reduction in the tissue contents of G-6-P and F-6-P. In addition, mannose treatment resulted in a marked decrease in the ATP content of the tissue. Although the ADP and AMP

contents of the slices generally increased during the treatment, the total increase in these two nucleotides was not equivalent to the measured loss of ATP.

TABLE 4. EFFECT OF INCUBATION OF SCUTELLUM SLICES IN MANNOSE ON THEIR CONTENTS OF G-6-P, F-6-P AND ADENINE NUCLEOTIDES*

Compound assayed	Content of tissue (μmoles/g fresh wt.)		
	Freshly prepared slices	After 1 hr incubation in:	
		Water	Mannose
Experiment 1			
G-6-P	0·97	0·74	0·07
F-6-P	0·18	0·19	<0·05
ATP	0·40	0·36	<0·05
ADP	0·12	0·18	0·24
AMP	0·08	<0·05	0·12
Experiment 2 (duplicate flasks were run where shown)			
G-6-P	1·09	0·68	0·06
	1·17	—	0·06
F-6-P	0·23	0·17	<0·05
	0·24	—	<0·05
ATP	0·46	0·42	<0·05
	0·50	—	0·06
ADP	0·23	0·24	0·29
	0·23	—	0·24
AMP	0·06	0·06	0·15
	0·05	—	0·12

* The slices (1·0 g fresh wt.) were incubated for 1 hr at 30° in either water or 0·05 M mannose after which they were washed to remove excess free sugars and extracted by grinding in cold 10% TCA. Extracts were prepared and analyzed by procedures given in the Experimental section.

DISCUSSION

The observations made during this study strongly support the idea that two distinct mechanisms for sucrose storage are found in the parenchymatous cell of the corn scutellum. One mechanism, which is operative between the bathing medium and the storage compartment (Fig. 1, arrow 3) is involved in the storage of exogenous sucrose and has been shown to be strongly inhibited by mannose. The second mechanism is involved in sucrose storage when the tissue slices are supplied exogenous hexose (fructose in these studies). Our data suggest that free sucrose is formed in the synthesis compartment from exogenously supplied hexose, and that the disaccharide is transported from the synthesis compartment into the storage compartment by an intercompartmental storage mechanism (Fig. 1, arrow 1). In contrast to the storage of exogenous sucrose, the storage of newly synthesized sucrose of the synthesis compartment is not inhibited by the presence of mannose (Tables 1 and 2). Thus, the two mechanisms for sucrose storage differ not only in their site within the cell but also in their capacity for transport in the presence of mannose.

Although mannose had no discernible effect on the storage of newly synthesized sucrose by the intercompartmental storage mechanism (Fig. 1, arrow 1), the synthesis of the disaccharide from exogenously supplied fructose was strongly inhibited. The data presented in

this paper do not allow us to give an unequivocable explanation for this inhibition; however, it is likely that the inhibition of sucrose synthesis results from a strong inhibition of glycolysis and the production of "glycolytic ATP". Inasmuch as mannose is a substrate for corn scutellum hexokinase,[9] the inhibition may result from a competitive interaction of mannose and fructose for this enzyme. In addition, the accumulation of large amounts of M-6-P in the tissue[6] may result in the inhibition of phosphoglucose isomerase. The competitive inhibition of phosphoglucose isomerase by M-6-P has been reported for honey bee,[10] *Euglena*[11] and tomato root.[12] However, M-6-P does not inhibit corn scutellum hexokinase.[9] Furthermore, mannose treatment may inhibit glycolysis by depleting the tissue of inorganic phosphate during the production of non-metabolizable M-6-P. Such an inhibition is also thought to occur when tomato roots are treated with mannose.[12] In any event, treatment of scutellum slices with mannose reduced the tissue level of ATP without a concomitant increase in ADP and AMP (Table 4). This may indicate that deamination of AMP or adenosine has occurred. A similar situation was found in yeast treated with 2-deoxyglucose.[13]

The above observations imply that the mechanism for storage of exogenous sucrose (Fig. 1, arrow 3) is in some manner dependent on a supply of ATP while the mechanism for intercompartmental storage is not. It may be that an active transport by an ATP-dependent carrier occurs between the external solution and the storage compartment, and that a reduction in the ATP level of the tissue results in a breakdown of this storage mechanism. Alternatively, the sucrose carriers of the storage membrane may be functional only if the membrane is maintained in a particular state or condition, and this functional state may require a continuing supply of ATP.

That ATP apparently is not required for sucrose storage via the intercompartmental mechanism and that sucrose phosphate was not found in the tissue slices does not necessarily mean that sucrose phosphorylation does not occur prior to the storage of accumulated free sucrose of the synthesis compartment. However, it seems probable that if sucrose phosphorylation does occur, it cannot result from an ATP-requiring, sucrose kinase reaction. It is possible that sucrose, as in the case of certain hexoses, may accept phosphate from a nonnucleotide donor. In this regard, Kundig *et al.*[14] have isolated a bacterial phosphotransferase system which catalyzes the transfer of phosphate from phosphoenolpyruvate to a number of carbohydrates, and Kaback[15] has shown this phosphoenolpyruvate phosphotransferase system to have a role in the transport of certain sugars by isolated membrane preparations of *Escherichia coli*. It is possible that a similar system may give rise to sucrose phosphate in the corn scutellum and that it is in this form that accumulated free sucrose is transported into storage. Accumulation of free sucrose in the storage volume of the cell would require the hydrolysis of the phosphate ester. Thus, the actual tissue level of sucrose phosphate could be well below our limit of detection.

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

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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 1·0 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 (1·0 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.[3]

Measurement of Metabolic Intermediates

The tissue levels of G-6-P, F-6-P and free adenine nucleotides were determined following the extraction of the tissue slices with cold TCA. The preparation of TCA extracts and the measurements of G-6-P, F-6-P and ATP were conducted by methods previously described.[5]

ADP was measured by determining the oxidation of $NADH_2$ in the presence of the extract and appropriate amounts of pyruvic kinase and lactic dehydrogenase. In addition to the scutellum extract and the enzymes, the reaction mixture (3·1 ml) contained 33 mM glycylglycine buffer (pH 7·5), 67 mM KCl, 6·7 mM $MgCl_2$, 0·17 mM $NADH_2$ and 0·31 mM phosphoenolpyruvate (trisodium salt). The decrease in absorbance (A) at 340 nm resulting from $NADH_2$ oxidation was measured.

AMP was determined by an adaptation of the above procedure for ADP. In addition to all of the above components, the reaction mixture contained 0·03 mM ATP. After the addition of pyruvic kinase and lactic dehydrogenase, the oxidation of $NADH_2$ was followed until a constant reading was achieved on the spectrophotometer. At this point, an appropriate amount of adenylate kinase was added to the cuvette. The oxidation of $NADH_2$ following the addition of adenylate kinase was determined and used to calculate the values for AMP.

The sucrose contents of the bathing solutions and ethanolic extracts of the tissue slices were determined by analyzing these solutions for glucose before and after invertase treatment. Glucose was determined by the glucose oxidase method.

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