Phytochemistry, 1968, Vol. 7, pp. 701 to 713. Pergamon Press. Printed in England

THE STORAGE OF EXOGENOUS SUCROSE BY CORN SCUTELLUM SLICES*

T. E. HUMPHREYS and L. A. GARRARD

Department of Botany, Agricultural Experiment Station, University of Florida, Gainesville, Florida

(*Received* 15 *November* 1967)

Abstract—Corn scutellum slices stored sucrose when incubated in either sucrose or fructose, and similar maximum rates of net storage were obtained with 0·4 M sucrose or 0·2 M fructose. It is concluded that exogenous sucrose is stored without prior inversion. This conclusion is based on the following: (1) The amount of extracellular inversion (as measured by the appearance of glucose and fructose in a 0·3 M sucrose bathing solution) was too low to support a net sucrose synthesis and storage, and (2) sucrose storage in slices bathed in optimal concentrations of fructose or glucose was increased by the addition of sucrose to the bathing medium. Further evidence for the existence of a transport system for exogenous sucrose was obtained in the demonstration of an exchange of sucrose between the bathing solution and the storage compartment. The rate of exchange was increased as the concentration of exogenous sucrose was increased, was doubled in the presence of citrate–phosphate buffer, was little affected by pH in the range 5·0–7·3 and showed no dependence on the net amount of sucrose stored.

INTRODUCTION

SLICES of the corn scutellum take up glucose or fructose at a rapid rate, and there is in each case an accumulation of sucrose in the tissue.[1,2] It appears that hexoses readily penetrate into areas of the cell where the synthesis of sucrose or a sucrose derivative occurs. Accumulation of sucrose involves the movement of sucrose or a sucrose derivative into a storage space and is not obligatorily coupled with the synthesis of the disaccharide.[2] During incubation of corn scutellum slices in fructose solutions, sucrose leaks from the tissue (leakage A) and a further leakage occurs when the fructose bathing medium is replaced with water (leakage B).[2] The results of these previous experiments suggest that leakage B sucrose is newly synthesized sucrose which is not stored during the incubation period in fructose but rather remains in the synthesis compartment, while leakage A comes from a sucrose storage pool and during incubation is released to the cell exterior by an exchange reaction at the storage compartment membrane. Sucrose leakage B is strongly inhibited by fructose and polyhydric alcohols[2] and calcium ion.[3] During inhibition of sucrose leakage B by fructose or mannitol, sucrose is neither stored nor utilized but remains in the synthesis compartment of the cell from which it leaks only after removal of the inhibitor.[2] During inhibition of leakage B by Ca^{2+}, the sucrose of the synthesis compartment is rapidly stored.[3] The possibility exists that during inhibition of leakage B by Ca^{2+} the sucrose held in the synthesis compartment is moved to the storage area(s) of the cell as sucrose rather than first being degraded to hexose which might be used to synthesize a transportable sucrose derivative (perhaps sucrose phosphate).

* Florida Agricultural Experiment Stations Journal Series, No. 2781. This investigation was supported, in part, by a research grant (No. AM 07299-05) from the National Institute of Arthritis and Metabolic Diseases, U.S. Public Health Service.

[1] T. E. HUMPHREYS and L. A. GARRARD, *Phytochem.* **3**, 647 (1964).
[2] T. E. HUMPHREYS and L. A. GARRARD, *Phytochem.* **5**, 653 (1966).
[3] L. A. GARRARD and T. E. HUMPHREYS, *Phytochem.* **6**, 1085 (1967).

The present investigation is a study of sucrose storage by corn scutellum slices using either sucrose or fructose as the exogenous sugar. It is clear that scutellum slices rapidly store sucrose when supplied an exogenous source of the disaccharide, and that movement of the sugar into the storage area(s) of the cell is not dependent on inversion and resynthesis.

RESULTS

Exogenous Hexose Compared to Exogenous Sucrose as a Sugar Source for Sucrose Storage

When scutellum slices were incubated in either sucrose or fructose there was in each case a net storage of sucrose (Table 1). The term "net storage" refers to the increase in sucrose content of the slices after incubation in sugar followed by a 30 min incubation period in water to remove the leakable sucrose. After incubation of the slices in fructose solutions, this leakable sucrose (leakage B, Table 1) came from an intracellular compartment and was

TABLE 1. SUCROSE LEAKAGE AND STORAGE WITH INCUBATION OF CORN SCUTELLUM SLICES IN SUCROSE OR FRUCTOSE*

Bathing medium	Sucrose (μmoles from 1 g fresh wt.)			
	Leakage A	Net storage	Total storage	Leakage B
Sucrose, 0·4 M	a†	58·4	58·4 + a	(32·2)
Fructose, 0·2 M	21·0	51·0	72·0	6·2

* The slices (1·0 g fresh wt.) were incubated at 30° for 3 hr in 10 ml of either sucrose or fructose solution after which the bathing medium was removed by suction and the slices were washed by the rapid addition and removal of two 10 ml portions of distilled water. The slices were then incubated in 10 ml of distilled water at 30° for 30 min after which the bathing medium was removed and a sample retained for the determination of sucrose leakage B. The tissue slices were immediately killed and extracted with hot 80 per cent ethanol for the determination of tissue sucrose. A portion of the initial bathing medium containing fructose was taken for the determination of sucrose leakage A.

† Sucrose leakage A could not be determined when sucrose was used in the bathing medium.

newly synthesized sucrose which was not stored.[2] After incubation in sucrose, the amount of sucrose which leaked from the slices into water reached a maximum value within 30 min. It is possible that a portion of this sucrose leaked from the B compartment (sucrose synthesis compartment) after having penetrated this part of the cell during the initial incubation period. In addition, an undetermined amount of sucrose leaked from intercellular spaces during the final incubation period in water. For this reason, the value for leakage B after sucrose incubation is placed in brackets (Table 1). In any event, that sucrose remaining in the tissue after the 30 min incubation in water is considered stored sucrose.

Leakage A sucrose is stored sucrose which leaks from the storage compartment into the fructose bathing medium during the initial incubation period in the hexose.[2] Since this is a loss of stored sucrose, this amount must be added to the net storage value to give total sucrose storage (Table 1). Whether or not leakage A occurs when the slices are incubated in sucrose cannot be determined. For this reason, values for net storage are given in this paper along with, in the case of fructose incubation, values for leakage A.

The amounts of sucrose stored with time when exogenous sucrose or fructose served as a sugar source are shown in Fig. 1. The rates of net sucrose storage were similar for the two sugar sources, but the concentrations of exogenous sugar necessary to obtain these rates were different. In previous experiments,[2] the highest rates of sucrose synthesis and storage were obtained using exogenous fructose at 0·2 M while in these experiments 0·4 M exogenous sucrose was required for maximum storage (Fig. 2). If the slices were incubated in sucrose at concentrations higher than 0·4 M there was a decrease in the amount of sucrose stored. Similarly, as the fructose concentration of the bathing medium is increased above 0·2 M there is a decrease in sucrose storage although sucrose synthesis is not affected.[2]

The effect of pH on the storage of sucrose by tissue slices incubated in sucrose or fructose solutions is shown in Fig. 3. When sucrose was provided in the bathing medium, the storage of sucrose by the tissue was strongly and progressively inhibited as the pH of the external solution was increased from 4·5 to neutrality. In contrast, the pH of the bathing medium had only a slight effect on the storage of sucrose by tissue slices incubated in fructose. This may indicate that the sites of sucrose uptake are in contact with the bathing medium and are affected by pH. Fructose, on the other hand, readily enters the cell where sucrose synthesis and storage take place in an environment little affected by the external pH.

An alternative explanation for the effect of pH on sucrose storage during incubation of tissue slices in sucrose solutions should be considered. A number of investigators have shown that sucrose uptake by various plant tissues is preceded by sucrose hydrolysis at a site exterior to the cell with the resulting hexoses being absorbed and utilized within the cell.[4–7] Convincing evidence has been presented that the storage of sucrose in sugar cane internodes involves the movement of sucrose from conductive tissue into the outer space (free space or cell wall zone) of adjacent parenchymatous tissue where the disaccharide is inverted by an acid invertase. The resulting hexoses are absorbed into a metabolic compartment where they are utilized to form a sucrose derivative (perhaps sucrose phosphate) which is necessary for the accumulation of free sucrose in the storage compartment.[6, 8–10] A portion of the evidence presented to support the essential nature of this outer space invertase in the sucrose accumulation process in sugar cane was the close similarity of the effect of pH on invertase activity and sucrose accumulation when sucrose was supplied as an exogenous sugar source. There was no correlation, however, between invertase activity and sucrose accumulation when glucose was supplied as the exogenous sugar source (see Fig. 2, Ref. 6). It is interesting that the effects of pH on sucrose accumulation in corn scutellum slices and in sugar cane internode discs are quite similar in that sucrose accumulation from exogenous sucrose is at a maximum at pH values near 4·5–5·0 with a progressive decrease in the amount of sucrose storage as the pH is increased to neutrality. In addition, sucrose storage from exogenous hexose by the two tissues was little affected by pH within the range 5·5–7·5 (cf. Fig. 3, this paper, with Fig. 2, Ref. 6). In view of these similarities, the question arises whether or not sucrose uptake by corn scutellum slices depends on the inversion of sucrose by an outer space, acid invertase as in the case of sugar cane internode tissue. This possibility was tested by measuring the hexose content of the bathing medium during incubation of

[4] E. W. Putnam and W. Z. Hassid, *J. Biol. Chem.* **207**, 885 (1954).
[5] J. L. Harley and D. C. Smith, *Ann. Bot. (London)* **20**, 513 (1956).
[6] J. A. Sacher, M. D. Hatch and K. T. Glasziou, *Plant Physiol.* **38**, 348 (1963).
[7] J. S. Hawker and M. D. Hatch, *Physiol. Plantarum* **18**, 444 (1965).
[8] M. D. Hatch and K. T. Glasziou, *Plant Physiol.* **38**, 344 (1963).
[9] M. D. Hatch, J. A. Sacher and K. T. Glasziou, *Plant Physiol.* **38**, 338 (1963).
[10] M. D. Hatch, *Biochem. J.* **93**, 521 (1964).

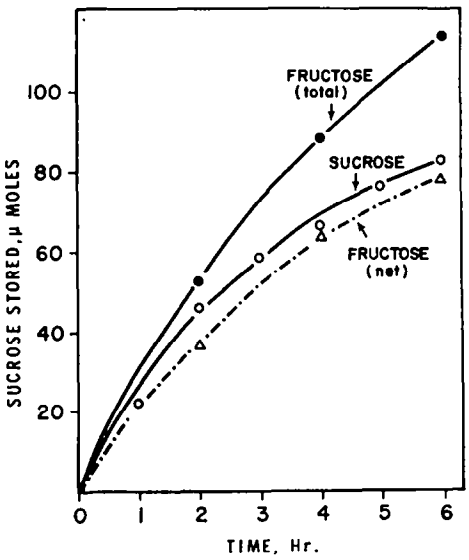

FIG. 1. SUCROSE STORED WITH TIME OF INCUBATION OF SCUTELLUM SLICES IN 0·2 M FRUCTOSE OR 0·4 M SUCROSE.

The slices (1 g fresh wt.) were incubated at 30° in 0·2 M fructose or 0·4 M sucrose for the periods shown. The slices were then washed twice with 10-ml portions of water followed by a 30 min incubation in 10 ml of water. After the water incubation they were killed and extracted in ethanol. At the end of each incubation period in fructose, a portion of the bathing solution was removed for the determination of sucrose leakage A. The "fructose (net)" curve shows the increase in sucrose stored in the tissue during incubation in fructose. The "fructose (total)" curve was obtained by adding the values of sucrose leakage A to the values of the "fructose (net)" curve.

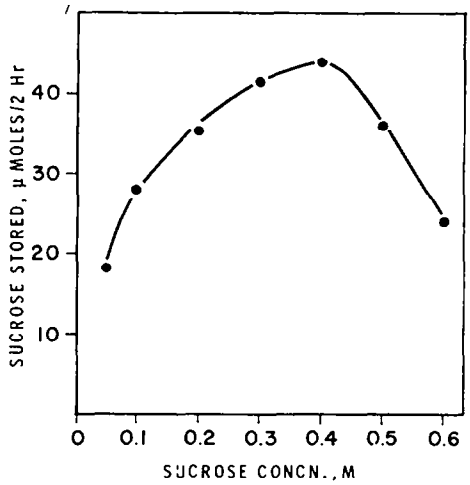

FIG. 2. SUCROSE STORED AS A FUNCTION OF THE SUCROSE CONCENTRATION OF THE BATHING SOLUTION.

The slices (1·0 g fresh wt.) were incubated at 30° for 2 hr in 10 ml of sucrose at the concentrations shown. At the end of the incubation period, the slices were washed twice with 10-ml portions of distilled water, and were placed in 10 ml of water for an additional 30 min incubation to remove leakable sucrose (unstored sucrose). Following this second incubation period, the slices were washed briefly on a small Buchner funnel and killed and extracted with hot 80 per cent ethanol.

scutellum slices in sucrose (Table 2) and by measuring the ability of the tissue to manufacture and store sucrose when incubated in hexose solutions at concentrations near those found in the sucrose-containing bathing medium (Table 3). During incubation of tissue slices in

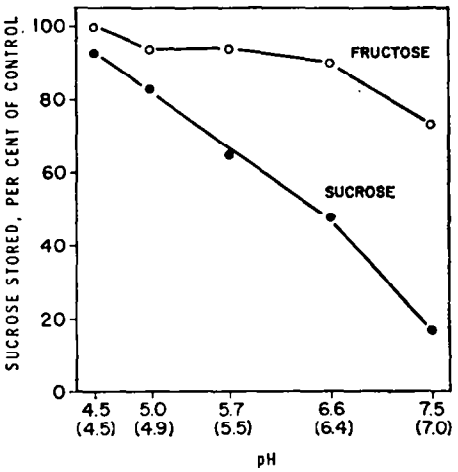

FIG. 3. THE EFFECT OF pH ON SUCROSE STORAGE.

The slices (1 g fresh wt.) were incubated at 30° in 0·1 M fructose or 0·3 M sucrose with each sugar solution containing 0·02 M potassium phosphate buffer at the pH shown. At the end of each 30 min period during the 2 hr incubation, the bathing solutions were removed and replaced with fresh solutions. In this way the pH was approximately maintained using dilute buffers. The actual pH at the end of the 30 min periods is shown in brackets below the initial pH. Following the 2 hr incubation period, the leakable (unstored) sugars were removed and the stored sucrose extracted from the tissue slices (see footnote, Fig. 2).

TABLE 2. HEXOSE CONTENT OF THE BATHING SOLUTION WITH LENGTH OF TIME THE SCUTELLUM SLICES WERE INCUBATED IN 0·3 M SUCROSE*

Incubation period (hr)	Hexose content (μmoles)		Hexose concn (mM)
	Glucose	Fructose	
1	8·3	7·4	1·57
2	13·8	12·9	2·67
3	16·4	17·3	3·37

* The slices (1·0 g fresh wt.) were incubated at 30° for the periods shown and then the bathing solution was removed and analyzed for hexose. Glucose was determined by following the reduction of NADP in the presence of hexokinase, ATP, $MgCl_2$ and G-6-P dehydrogenase. Fructose was determined with the same system plus phosphohexose isomerase.

sucrose, small quantities of hexose appeared in the ambient solution. The concentration of hexose increased with time of incubation, and the ratio of glucose to fructose in the bathing medium remained close to unity throughout the incubation period (Table 2). These results suggest that some inversion of sucrose did occur during the incubation period. However, evidence that sucrose inversion in the tissue outer space is not an essential feature of the

sucrose uptake process was obtained. If the hexose concentration of the sucrose-containing bathing solution is an accurate measure of the amount of hexose present at sites of sugar uptake, then these amounts could not support sucrose synthesis and storage by the tissue. Although hexose at low concentrations was rapidly taken up by the slices, the sucrose content was essentially unchanged. Glucose at a concentration of 0·01 M was utilized by the slices at a rate of about 20 μmoles/hr/g fresh wt. (Table 3). A high rate of alcoholic fermentation triggered by the addition of glucose can account for the major portion of the glucose utilized (unpublished data). If, on the other hand, sucrose inversion results in concentrations of hexose which are substantially higher at the sites of uptake than in the ambient sucrose solution, it is quite unlikely that the glucose–fructose ratio of the bathing medium would be near unity. This observation is based on the fact that glucose strongly inhibited (30–50 per cent) the uptake of fructose by the slices when the two sugars were present in equimolar concentrations. Although the above data do not support a requirement for sucrose inversion in the outer space of the tissue prior to uptake, they do not rule out the possibility that the

TABLE 3. UTILIZATION OF GLUCOSE PRESENT IN LOW CONCENTRATIONS IN THE BATHING SOLUTION*

	Initial hexose content of bathing solution		Glucose content of bathing solution after 3 hr	Glucose utilized	Change in tissue sucrose
	Conc. (mM)	(μmoles)	(μmoles)	(μmoles)	(μmoles)
Glucose	5	50	13·6	36·4	−2·6
Glucose	10	100	32·8	67·2	+4·4
Glucose + Fructose	5 + 5	50 + 50	15·6	34·4	+1·6

* The slices (1·0 g fresh wt.) were incubated in the hexose solutions at 30° for 3 hr. At the end of this period a portion of the bathing solution was removed for glucose and sucrose determinations and the slices were killed and extracted with 80 per cent ethanol.

disaccharide is degraded to hexose during the transport across the plasma membrane or at some site within the cell (although outside the storage compartment) and that resynthesis is a requirement for storage.

If the storage of exogenous sucrose entails splitting of the disaccharide to free hexose (whether during transport across the cell membrane or at some site within the cell), then the addition of sucrose to tissue slices supplied with sufficient hexose to produce maximum sucrose storage should not result in additional sucrose storage. The results of such an experiment are shown in Table 4. In this experiment, the scutellum slices were incubated in water for 1 hr prior to the addition of the sugars because this was found to decrease leakage A, and thus less uncertainty was introduced in the values for total sucrose stored (net storage plus leakage A). The addition of sucrose to the bathing solutions containing either glucose or fructose resulted in increased sucrose storage (Table 4). This increase was greater than that obtained when glucose was added to fructose or vice versa. It is concluded from these data that the process of storage of exogenous sucrose can occur without free hexoses as intermediates.

When the bathing solution contained a single hexose, sucrose production and sucrose storage were about equal (leakage B was small, Table 4), but the addition of the second hexose

increased sucrose production more than sucrose storage (leakage B increased). It appears, therefore, that there was a saturation of the transport system responsible for the storage of newly synthesized sucrose. Nevertheless, the addition of sucrose to the bathing solution resulted in an increased rate of storage. This may indicate either that there are transport sites available for exogenous sucrose which are not available to newly synthesized sucrose or that the transport mechanisms for newly synthesized sucrose and exogenous sucrose are different in nature. It must be noted here that the amount of net storage when both 0·2 M fructose and 0·1 M sucrose were present in the bathing medium was less than the sum of the net storage values for each sugar taken separately while, in the case of 0·2 M glucose

TABLE 4. STORAGE OF SUCROSE IN CORN SCUTELLUM SLICES INCUBATED WITH HEXOSE OR HEXOSE PLUS SUCROSE*

Bathing solution	Sucrose (μmoles from 1 g fresh wt.)			
		Leakage		
	Net storage	A	B	Total produced
Fructose (0·2 M)	70·8	7·5	3·3	81·6
Fructose (0·2 M)+glucose (0·1 M)	77·8	a†	13·9	91·7+a
Fructose (0·2 M)+sucrose (0·1 M)	93·1	—	—	—
Glucose (0·2 M)	61·1	a	5·6	66·7+a
Glucose (0·2 M)+fructose (0·1 M)	75·0	a	18·3	93·3+a
Glucose (0·2 M)+sucrose (0·1 M)	99·2	—	—	—
Sucrose (0·1 M)	40·2	—	—	—

* The slices (1·0 g fresh wt.) were incubated at 30° for 1 hr in 10 ml of water. The water was removed by suction, the slices were washed with 10 ml of water and then 10 ml of the above sugar solutions were added. The slices were incubated in the sugar solutions at 30° for 3 hr. At the end of this period the sugar solution was removed, the slices were washed twice with 10 ml of water and then 10 ml of water were added. The slices were incubated for an additional ½ hr and then a portion of the bathing solution was removed for the determination of leakage B, after which the slices were killed and extracted with 80 per cent ethanol. The slices from one flask were killed at the end of the first water incubation. The sucrose content of these slices served as a control to calculate net storage. A portion of the bathing solution from the 0·2 M fructose flask was removed at the end of the 3 hr incubation for the determination of sucrose leakage A.

† Sucrose leakage A could not be determined when sucrose was used in the bathing medium and was not determined when glucose was present.

and 0·1 M sucrose, the amount of net storage was approximately equal to the sum of the separate amounts. In either case, when hexose and sucrose were present together, the net sucrose stored was about the same (93·1 and 99·2 μmoles) so that the full transport capacity of the tissue may have been reached.

The above results, taken together, strongly support the existence of a transport system for exogenous sucrose. Further evidence for this was obtained in the demonstration of sucrose exchange.

Sucrose Exchange

To demonstrate sucrose exchange, the scutellum slices were incubated in dilute ^{14}C-sucrose in order to label the sucrose of the storage compartment. Following this initial

incubation, the slices were washed three times in 10-ml portions of water and incubated in 10 ml of water for an additional 30 min period to remove leakable ^{14}C-sugars thus ensuring that the labeled compounds remaining in the tissue were in the storage compartment. After

TABLE 5. DISTRIBUTION OF ^{14}C AMONG SUGARS IN ETHANOL EXTRACTS OF CORN SCUTELLUM SLICES*

	Extract 1 (cpm)	Extract 2 (cpm)
Total ethanol extract	244,000	248,000
Glucose	6,522	13,187
Fructose	5,435	14,286
Sucrose	202,174	222,222
Per cent of total recovered as sucrose	83%	90%

* The slices (1·0 g fresh wt.) were placed in closed flasks which contained $1{\cdot}1 \times 10^{-2}$ M sucrose and 2·0 μc of [U-^{14}C] sucrose in a volume of 10 ml. The slices were incubated at 30° for 2 hr after which the bathing solution was removed, the slices were washed three times with 10-ml portions of water and 10 ml of water was added to the flask. After an additional 30 min incubation in water to remove excess ^{14}C, the slices were killed and extracted in 80 per cent ethanol. Portions of the extracts were chromatographed on paper, and the sugars were located, eluted and counted as described in the Experimental section of this paper.

TABLE 6. DISTRIBUTION OF ^{14}C AMONG SUCROSE AND THE HEXOSES IN THE BATHING SOLUTION*

	^{14}C (cpm) found in:			
Bathing solution	Complete solution	Sucrose	Fructose	Glucose
Buffer, pH 5·0	26	19	0	2
Same + 0·1 M sucrose	223	234	6	10

* The slices were loaded with [U-^{14}C] sucrose and the excess ^{14}C removed by washing and an additional 30 min incubation in water (see footnote, Table 5). The slices were washed again and were placed in 9·0 ml of either citrate–phosphate buffer (0·06 M, pH 5·0) or buffer plus 0·1 M sucrose. The slices were incubated in these solutions at 30° for 150 min and then 2·0 ml of the bathing solutions were removed. These aliquots were taken to dryness under reduced pressure and the residues taken up in 0·5 ml of 50 per cent ethanol. A portion of this ethanol solution (0·05 ml) was chromatographed on paper and the sugars located and eluted. The eluates were concentrated on a steam bath to give a final volume of 2·0 ml. A portion of this volume (0·2 ml or 1/450 of the initial bathing solution) was plated and counted. The cpm given are not corrected for self-absorption.

such treatment, 80–90 per cent of the ethanol-soluble ^{14}C of the tissue was present as sucrose (Table 5). When slices treated in the above manner were placed in unlabeled sucrose, ^{14}C appeared in the bathing solution, and the amount of ^{14}C released from the tissue increased as the concentration of unlabeled sucrose in the bathing medium was increased (Fig. 4).

As can be seen in Table 6, the ^{14}C released into the bathing solution was almost entirely in sucrose. In contrast with the results obtained using sucrose in the bathing medium, neither

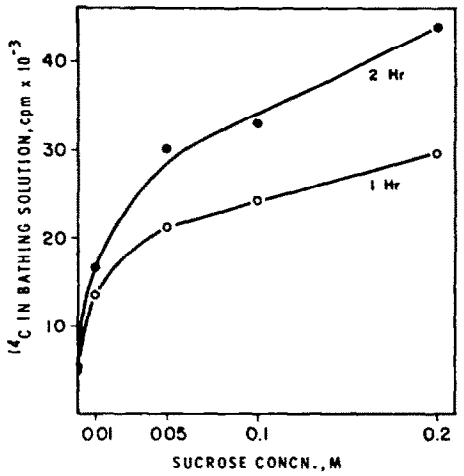

FIG. 4. AMOUNT OF ^{14}C LEAKING INTO BATHING SOLUTIONS OF DIFFERENT SUCROSE CONCENTRATIONS.

The slices (1 g fresh wt.) were loaded with ^{14}C-sucrose and the excess ^{14}C removed (see footnote, Table 5). The slices were washed again and placed in 9·0 ml of water or sucrose at the concentrations shown. The ^{14}C content of the bathing solutions was determined after 1 and 2 hr.

TABLE 7. EFFECT OF BUFFER AND pH ON SUCROSE EXCHANGE AND SUCROSE STORAGE*

Bathing solution	^{14}C in bathing soln. (cpm x 10^{-3})	Difference (sucrose exchange) (cpm x 10^{-3})	Change in tissue sucrose (μmoles)
Water (pH 3·7)	5·4		
Sucrose, 0·2 M (pH 3·7)	50·2	44·8	(+) 50·0
Buffer, pH 5·0	10·0		
Buffer, pH 5·0 + sucrose, 0·2 M	98·7	88·7	(+) 30·5
Buffer, pH 6·0	23·2		
Buffer, pH 6·0 + sucrose, 0·2 M	109·3	86·1	(+) 4·2
Buffer, pH 7·3	49·6		
Buffer, pH 7·3 + sucrose, 0·2 M	124·8	75·2	(−) 9·7

* The slices (1·0 g fresh wt.) were loaded with [U-^{14}C] sucrose and the excess ^{14}C removed (see footnote, Table 5). The slices were then placed in 9·0 ml of the solutions shown above and incubated for 150 min at 30°. The buffer used was 0·06 M citrate–phosphate. Following the incubation period, aliquots (0·1 ml) of the bathing solutions were taken for ^{14}C assay. The cpm given in the table are corrected for background and self-absorption. A separate set of slices was carried through the same procedure but with unlabeled sucrose, and these slices were killed and extracted for sucrose analysis.

glucose nor fructose were very effective in causing the release of ^{14}C from the tissue (Fig. 5). These results indicate that the exit of ^{14}C from the slices is not due to leakiness but is caused by sucrose exchange across the storage compartment membrane.

The exit of ^{14}C from the tissue slices, both in the presence and absence of exogenous sucrose, was increased as the pH of the bathing medium was increased from 3·7 (unbuffered solution) to 7·3 (Table 7). However, the actual exchange (the exit of ^{14}C-sucrose that could be attributed to the presence of the added unlabeled sucrose in the bathing medium) was little affected by pH, but the exchange in the presence of the buffers was approximately double that in the absence of buffer. The amount of exogenous sucrose stored bore no relation to the amount of sucrose exchanged (Table 7). As the pH of the bathing medium was increased from 5·0 to 7·3, the net amount of sucrose stored decreased to zero while the amount of sucrose exchanged remained nearly constant.

It is of interest that the use of citrate–phosphate buffer, pH 7·3, alone as the bathing medium resulted in a 10-fold increase in the amount of ^{14}C leakage as compared with the water control (Table 7). Furthermore, the leakage into water was complete in 40 min (Fig. 5)

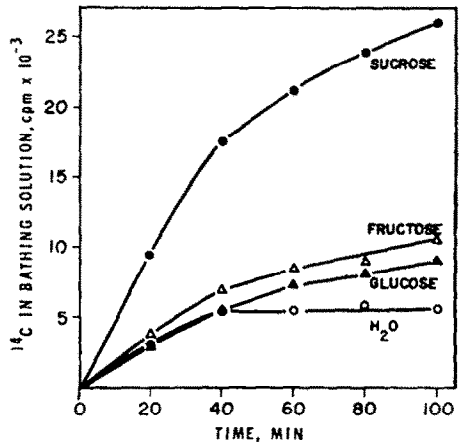

FIG. 5. INFLUENCE OF SUCROSE AND HEXOSE ON ^{14}C LEAKAGE.

The slices were loaded with ^{14}C-sucrose and the excess ^{14}C removed (see footnote, Table 5). The slices were placed in water or in 0·05 M sugar solutions. Aliquots of the bathing media were removed at the times shown for ^{14}C analysis.

while the leakage into the buffer (pH 7·3) continued throughout the 150 min experimental period. In addition to this effect in increasing leakiness of the tissue, raising the pH of the bathing solution to near neutrality also strongly inhibited the storage of exogenously supplied sucrose (Fig. 3 and Table 7). Both of these effects could be attributed to the effect of bathing solution pH on the storage compartment membrane and would suggest contact of the ambient solution with the membrane surface of the storage compartment.

DISCUSSION

During germination of the cereal grain, the starch of the endosperm is degraded to soluble sugars primarily by the action of alpha and beta amylases. Glucose of the endosperm is absorbed by the scutellum and converted to sucrose in which form it is transported.[11] Slices of the corn scutellum take up either glucose or fructose at a rapid rate, and there is a

[11] J. EDELMAN, S. I. SHIBKO and A. J. KEYS, *J. Exptl Botany* **10**, 178 (1959).

concomitant accumulation of sucrose.[1,2] Glucose and fructose do not accumulate within the tissue slices but rather enter an intracellular space (a synthesis compartment), the hexose content of which is in equilibrium with the solution bathing the tissue.[12] It is probable that the hexoses readily penetrate into areas of the cell where sucrose synthesis occurs, and that sucrose accumulation involves the transport of sucrose or a derivative of sucrose into a storage compartment. While high rates of sucrose synthesis and storage may be achieved utilizing exogenous hexose as a sugar source, these processes are not obligatorily coupled, and substantial amounts of newly synthesized sucrose may be free to leak from the synthesis compartment depending on the experimental conditions.[2] Sucrose of the synthesis compartment leaks freely into water; however, this leakage is strongly inhibited by Ca^{2+} in the ambient solution and, during the period of Ca^{2+}-inhibited leakage, the sucrose of the synthesis compartment is moved to the storage compartment.[3] The mechanism involved in the storage of sucrose from the synthesis compartment under the conditions outlined above remains obscure and several possibilities come to mind. These possibilities include: (1) the sucrose may be degraded to hexoses (perhaps by inversion) and stored after resynthesis of sucrose or a derivative of sucrose, (2) a derivative of sucrose is directly formed from sucrose without degrading to hexose and this compound is transported to the storage compartment, and (3) there are sites at the storage compartment membrane where unaltered sucrose may be transported into the storage compartment.

While ample evidence is available to support the essential nature of sucrose hydrolysis (inversion) to its uptake by a number of tissues (Canna leaves,[4] thalli of *Peltigera polydactyla*,[5] immature sugar cane internode tissue[6] and mature sugar cane internode tissue[7]) the data from the present study are not consistent with the premise that sucrose uptake and storage by corn scutellum slices is dependent on extracellular inversion of the disaccharide. This is apparent when one considers that the extent of inversion of exogenous sucrose would not support sucrose synthesis and storage, and that exogenous sucrose increased sucrose storage above the level obtained when scutellum slices were synthesizing and storing sucrose at maximal rates from exogenous hexose (Tables 2, 3 and 4). The fact that exogenous sucrose may be taken up unaltered by plant tissues has been reported previously. In this regard, Porter and May[13] found that sucrose (asymmetrically labeled with ^{14}C) was unaltered during its accumulation in tobacco leaf discs, and Kriedemann and Beevers[14] presented evidence along several lines which led them to conclude that by far the greater part of sucrose absorbed by castor bean cotyledons was not hydrolyzed prior to or during uptake. In addition, Sacher[15] found that sucrose may be taken up and accumulated by bean pod tissue without first being hydrolyzed, and he concluded that the absence of competition among hexoses and sucrose indicated that a common carrier was not involved in their uptake.

The demonstration of an exchange of sucrose between the storage compartment of corn scutellum cells and the bathing solution indicates the existence of a carrier capable of transporting sucrose across the storage compartment membrane. Park[16] refers to this type of exchange as "exchange diffusion" which is postulated to result when a carrier can cross a membrane only in a complex with its substrate. Thus, no net transport takes place but exchange between substrate molecules on opposite sides of the membrane would occur. It

[12] L. A. GARRARD and T. E. HUMPHREYS, *Nature* **207**, 1095 (1965).
[13] H. K. PORTER and L. H. MAY, *J. Exptl Botany* **6**, 43 (1955).
[14] P. KRIEDEMANN and H. BEEVERS, *Plant Physiol.* **42**, 174 (1967).
[15] J. A. SACHER, *Plant Physiol.* **41**, 181 (1966).
[16] C. R. PARK, in *Membrane Transport and Metabolism* (edited by A. KLEINZELLER and A. KOTYK), p. 20. Academic Press, New York (1961).

is supposed that such a phenomenon is an aspect of a more complex system capable of net transport.

The rate of exchange between ^{14}C-sucrose of the storage compartment of corn scutellum cells and unlabeled sucrose of the ambient solution surrounding the tissue would depend, among other things, on the number of sucrose carrier sites available and the specific activity of the sucrose-carrier molecules. The storage of ^{12}C-sucrose would decrease the specific activity of the labeled sucrose of the storage compartment which in turn would be expected to result in a decrease in the rate of ^{14}C-sucrose exchanged as measured by its appearance in the ambient solution. However, as can be seen in the results given in Table 7, this decrease in exchange did not occur. In this experiment the slices contained 64 μmoles of sucrose/g at the beginning of the exchange period when the buffers (pH 5·0 or 6·0) plus 0·2 M sucrose were added. At the end of the experiment, the slices at pH 5·0 contained 94 μmoles of sucrose while those at pH 6·0 contained 68 μmoles. In spite of the greater dilution of the ^{14}C-sucrose in the slices incubated at pH 5·0, the amount of exchange was essentially the same in both cases. It is concluded that the increase in concentration of stored sucrose compensated for the decrease in specific activity either by increasing the number of sucrose-carrier sites or by increasing the rate of sucrose exchange at the storage compartment membrane.

Since the storage of exogenous sucrose was strongly influenced by the pH of the bathing solution while the storage of newly synthesized sucrose produced inside the cell was not significantly influenced, and since sucrose exchange was doubled by the presence of citrate–phosphate buffer in the bathing solution, we suggest that the storage compartment membrane is accessible to the bathing medium and that sucrose can move into storage directly from the ambient solution. In this regard, we concluded from previous work[2] that leakage A sucrose comes from the storage compartment and leaks into the bathing solution without passing through the sucrose synthesis compartment of the cell. Our previous studies[2] also indicate that sucrose exchange occurs between the synthesis compartment and the storage compartment. Thus, the two phenomena, storage and exchange, occur both between the storage compartment and the sucrose synthesis compartment and between the storage compartment and the bathing solution.

In studies where ^{14}C-sugars are used to measure uptake, the occurrence of sugar exchange could cause a large over-estimate of net sugar uptake. In addition, the existence of sucrose exchange would have a bearing on the interpretation of data from experiments utilizing asymmetrically ^{14}C-labeled sucrose to determine the relevance of sucrose inversion to sucrose uptake. When, for example, (fructosyl-^{14}C)-sucrose is fed to a tissue, it could appear (as the result of sucrose exchange) that there was a net uptake of sucrose which entered the tissue with little inversion although this might not actually be the case. We do not know whether sucrose exchange occurs in other tissues capable of taking up sucrose unaltered. However, Glasziou[17] was unable to demonstrate a sucrose exchange in discs of sugar cane internode tissue, a tissue in which inversion of sucrose is a prerequisite to uptake.

In corn scutellum slices bathed in 0·1 M fructose, there is very little sucrose outside the storage compartment indicating that sucrose storage takes place as rapidly as synthesis.[2] This means that either the storage mechanism has a high affinity for sucrose or that free sucrose is not an intermediate in the transport scheme. It may be, as Hatch[10] proposes for sugar cane, that sucrose phosphate is the intermediate involved in sucrose storage. In the case of the storage of exogenous sucrose, however, the production of sucrose phosphate is not so likely. A sucrose kinase could be invoked, but if sucrose exchange is an aspect of the

[17] K. T. GLASZIOU, *Plant Physiol.* **35**, 895 (1960).

mechanism for net uptake, then it would appear that the kinase would have to be the transmembrane carrier. Alternatively, sucrose exchange may be associated with a mechanism for sucrose exit from storage rather than having a role in sucrose uptake.

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 9·0 or 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]

In experiments requiring the incubation of tissue slices in [U-^{14}C]-sucrose (Nuclear-Chicago Corp.) all vessels were equipped with center wells containing 0·2 ml of 10 per cent KOH. The slices were incubated in closed flasks which contained $1{\cdot}1 \times 10^{-2}$ M sucrose and 2·0 μc of ^{14}C-sucrose.

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.).

The ^{14}C contents of the hexoses and sucrose of the bathing medium and ethanolic extracts of the tissue slices were determined following separation of the sugars by paper chromatography. Portions of the bathing solutions and extracts were chromatographed descendingly on Whatman No. 1 paper in ethyl acetate–pyridine–water (8:2:1 v/v). The chromatograms were developed for a period of 24 hr at room temperature and dried under a warm flow of air. The sugars were located on the chromatograms by spraying marker strips with aniline-diphenylamine and were quantitatively eluted from the paper with 10 ml of boiling water followed by washing with three 10 ml portions of hot 80 per cent ethanol. The eluates were concentrated on a steam bath and centrifuged to remove paper fibers before aliquots were plated in stainless-steel planchets, dried and counted in a gas-flow counter. In each case, an amount of bathing solution or alcohol extract equivalent to that used for chromatographic separation was spotted and dried on Whatman No. 1 paper. This material was eluted from the paper and handled in the manner above without being chromatographed. Planchets prepared in this manner were used to determine the total count for the entire extract or bathing solution. Where warranted, corrections were made for self-absorption.

Acknowledgement—We thank Mr. Donald Winsor for his valuable assistance.