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LEAKAGE AND STORAGE OF SUCROSE DURING SUCROSE SYNTHESIS IN THE CORN SCUTELLUM*

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Abstract—During incubation of corn scutellum slices in fructose solutions (0·1–0·9 M) sucrose leaked (*leakage A*) from the slices, and additional leakage (*leakage B*) occurred upon dilution of the fructose solution to 0·1 M or less. Sucrose was also stored within the slices, and net sucrose synthesis (stored plus leaked) was 20–25 μmoles/hrg fresh wt. Fructose concentration of the bathing solution had little effect on net sucrose synthesis or on the amount of sucrose *leakage A*. However, with increased fructose concentration, the amount of sucrose stored decreased while the amount of sucrose *leakage B* increased. The rate of sucrose *leakage B* was strongly inhibited by fructose, galactose, polyhydric alcohols and 2,4-dinitrophenol. After incubation of the slices in ^{14}C-fructose, *leakage A* and *leakage B* sucrose were found to be quite different with respect to specific activity and distribution of ^{14}C between the fructose and glucose moieties. From these results and the results of inhibition studies, it is concluded that the two types of leakage have different origins within the cell.

INTRODUCTION

SLICES of the corn scutellum take up glucose at a rapid rate, and there is a concomitant accumulation of sucrose.[1] Glucose and fructose are not accumulated although the scutellum tissue contains an intracellular hexose space the hexose content of which is in equilibrium with the outside solution.[2] It appears, therefore, that hexoses readily penetrate into areas of the cell where synthesis of sucrose or a sucrose derivative occurs, and that sucrose accumulation involves the movement of sucrose or a sucrose derivative into a storage space (vacuole?). It has been suggested that sucrose phosphate is an intermediate in the sucrose accumulation process in sugar cane, and that accumulation involves the hydrolysis to free sucrose during or after transport into the storage compartment.[3,4] Our studies, presented in this paper, indicate that there is not an obligatory coupling between sucrose synthesis and sucrose storage in the corn scutellum.

RESULTS

Sucrose Leakage and Storage

During incubation of scutellum slices in fructose solutions there was a rapid net synthesis of sucrose amounting to 20–25 μmoles/hrg fresh wt. Some sucrose leaked from the slices into the bathing solution during incubation in fructose and additional leakage occurred upon dilution of the fructose bathing solution (Fig. 1). The amounts of sucrose leaked into

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[2] L. A. GARRARD and T. E. HUMPHREYS, *Nature* **207**, 1095 (1965).
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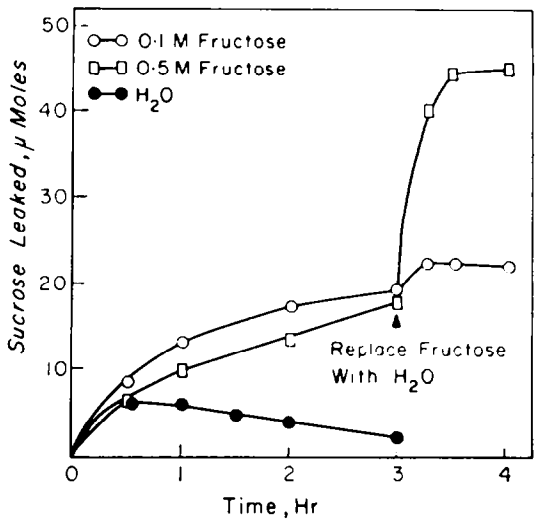

FIG. 1. THE EFFECT OF FRUCTOSE CONCENTRATION ON THE LEAKAGE OF SUCROSE FROM THE SCUTELLUM SLICES INTO THE BATHING SOLUTION.

At zero time, the slices (1 g fresh wt.) were placed in flasks containing 9·0 ml of water or fructose solution and were incubated at 30°. At the end of 3 hr, all but 2 ml of the fructose bathing solutions were removed and 8·0 ml of water were added to the flasks. At the times indicated in the graph, portions of the bathing solutions were removed for sucrose analysis.

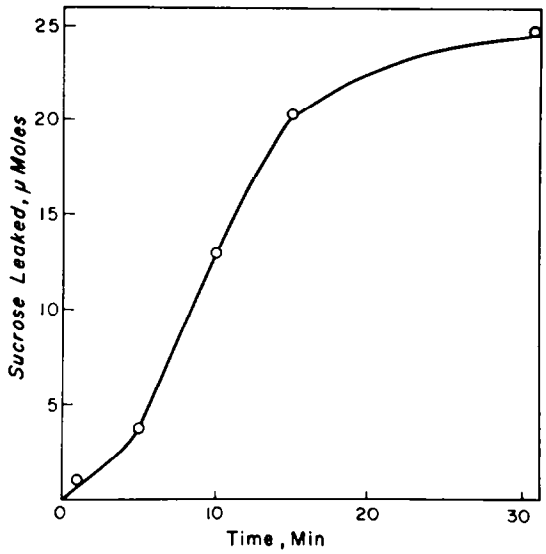

FIG. 2. THE TIME COURSE OF SUCROSE *leakage B* FOLLOWING INCUBATION OF SCUTELLUM SLICES IN 0·5 M FRUCTOSE.

The slices (1 g fresh wt.) were incubated at 30° for 3 hr in 9·0 ml of 0·5 M fructose. At the end of this period, 7·0 ml of the bathing solution were removed and 8·0 ml of water were added. Zero time in the graph was the time at which the 8·0 ml were added to vessel. Portions of the bathing solution were removed for sucrose analysis at the times shown. The sucrose values shown have been corrected by subtracting the sucrose content of the 2·0 ml of original bathing solution which remained in the flask (4–5 μmoles).

0·1 or 0·5 M fructose were approximately the same after 3 hr. However, when the fructose bathing solutions were diluted five-fold about eight times as much additional sucrose leaked from the slices which had been incubated in 0·5 M fructose as leaked from those incubated in 0·1 M fructose. Some sucrose leaked from slices which were incubated in water, but this leakage occurred during the first 30 min of incubation and the sucrose was rapidly reabsorbed. In what follows, it is convenient to refer to leakage into the initial, concentrated, fructose solution as *leakage A* and that which occurred after dilution of the fructose solution as *leakage B*.

The course of sucrose *leakage B* was measured at short time intervals to give the curve shown in Fig. 2. There was a lag period of approximately 5 min before the high, constant rate of sucrose leakage occurred. The presence of this lag period made it possible to carry out experiments which required certain manipulations prior to the measurement of *leakage B*.

TABLE 1. INCORPORATION OF ^{14}C-FRUCTOSE INTO SUCROSE OF CORN SCUTELLUM SLICES*

Sucrose source	Vessel‡	Sucrose content (μmoles from 1 g fresh wt.)	Specific activity of sucrose (counts/min μmole)	Ratio of ^{14}C-glucose to ^{14}C-fructose
Leakage A	1	21·8	4261	0·48
	2	18·9	3973	0·45
Leakage B†	1	26·0	3219	0·76
	2	28·3	3286	0·89
Tissue	1	157·8	3249	0·95
	2	153·5	3162	0·90

* The slices (1 g fresh wt.) were incubated at 30° for 3 hr in 9·0 ml of uniformly labeled ^{14}C-fructose (0·5 M, specific activity ~2860 counts/min μmole). At the end of the incubation period, 7·0 ml of the bathing solution (containing *leakage A* sucrose) were removed from each vessel and 8·0 ml of water were added. After an additional 30 min incubation, the bathing solutions (containing *leakage B* sucrose) were removed and the tissue slices killed and extracted with boiling 80% ethanol. The specific activities and ^{14}C distribution ratios were obtained from sucrose isolated by paper chromatography (see Experimental section).

† All values are corrected for sucrose (*leakage A*) remaining in the flasks at the time of addition of 8·0 ml of water to the vessels.

‡ Vessels 1 and 2 were duplicates.

For instance, slices which had been incubated in 0·5 M glucose for 3 hr were washed in running water for 5 min prior to measurement of sucrose *leakage B*. The removal of the great excess of glucose made it possible to measure sucrose leakage which was of about the same magnitude as that obtained after incubation in fructose (~ 25 μmoles).

In order to delineate the two types of sucrose leakage, the tissue slices were allowed to synthesize ^{14}C-sucrose during a 3-hr period of incubation in 0·5 M uniformly labeled ^{14}C-fructose. After the incubation period the fructose bathing solution was diluted five-fold and incubation continued for an additional 30-min period. The tissue sucrose and the sucrose of *leakages A* and *B* were analyzed for ^{14}C content and ^{14}C distribution in the hexose moieties of the sucrose. These results are shown in Table 1. The tissue sucrose and *leakage B* sucrose were quite similar with respect to the specific activity and distribution of ^{14}C indicating that these two sucrose pools are either one and the same or are in isotopic equilibrium. The ^{14}C distribution and specific activity of the *leakage A* sucrose, however, were quite different from the other two. A calculation based on the specific activity and ^{14}C distribution ratio

shows that the specific activity of the fructose moiety of the *leakage A* sucrose is the same as that of the bathing solution fructose. The levels of hexose phosphates and glucose in the tissue are too low to account for these results. Although these results suggest that *leakage A* sucrose did not come from the main sucrose pool, it is probable that the glucose moiety of *leakage A* sucrose did originate there. An exchange reaction between the unlabeled sucrose of the tissue and the ^{14}C fructose could account for the labeling pattern of the *leakage A* sucrose. This exchange could be catalyzed by uridine diphosphate-glucose-fructose glucosyltransferase and would occur at the membrane separating the tissue sucrose and the ^{14}C-fructose. Furthermore, following the exchange, some of the sucrose must be released on the

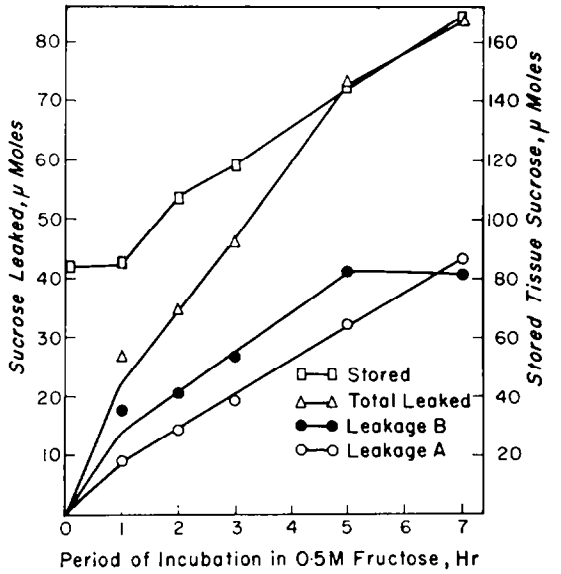

FIG. 3. SUCROSE STORAGE AND LEAKAGE WITH TIME OF INCUBATION IN 0·5 M FRUCTOSE.

The slices (1 g fresh wt.) were incubated at 30° in 9·0 ml of 0·5 M fructose for the periods shown. Duplicate flasks were prepared for each time period. The slices from one flask of each set were killed and extracted for determination of tissue sucrose. The other flask of each set was used to determine sucrose leakage as follows: At the end of the appropriate time period, 7·0 ml of the bathing solution were removed and used to determine sucrose leakage into 0·5 M fructose (*leakage A*). Then 8·0 ml of water were added to the vessel and, after an additional 1 hr incubation period, the sucrose content of the bathing solution was determined (*leakage B*). Stored sucrose was calculated by subtracting the quantity of sucrose in *leakage B* from the tissue sucrose for each time period.

fructose side of the membrane, perhaps due to the high fructose concentration there. On the other hand, if *leakage B* sucrose and the tissue sucrose occupy separate pools which are in isotopic equilibrium, the equilibrium must result from an exchange of sucrose molecules since an exchange involving only fructose would not result in the high ^{14}C distribution ratio.

Figure 3 shows the sucrose leaked and the amount of storage sucrose with time of incubation in 0·5 M fructose. In this case, storage sucrose is that part of the tissue sucrose which does not leak into 0·1 M fructose (i.e. tissue sucrose minus *leakage B* sucrose). However, it was shown in other experiments that the same amount of sucrose leakage (*leakage B*) occurred if the bathing solution was diluted to 0·02 M fructose or if the slices were quickly washed and placed in water. After the first hr of incubation in 0·5 M fructose the rate of *leakage A* and

the rate of sucrose storage were essentially constant. The production of *leakage B* sucrose reached a plateau after 5 hr. The amount of leakage (*A* plus *B*) and the amount of sucrose stored were approximately the same at the end of the 7-hr incubation period. The average net sucrose production was approximately 25 μmoles/hrg fresh wt. The fructose concentration of the bathing solution decreased during incubation as a result of fructose uptake by the tissue slices. However, it is estimated that after 7 hr this decrease amounts to only 10–15 per cent of the total bathing solution fructose. Since it is difficult to obtain accurate uptake measurements from concentrated sugar solutions, this estimate is based on uptake rates obtained with 0·1 M and 0·2 M concentrations of fructose or glucose. These sugars were

TABLE 2. EFFECT OF FRUCTOSE CONCENTRATION OF THE BATHING SOLUTION ON SUCROSE SYNTHESIS AND DISTRIBUTION WITHIN SCUTELLUM SLICES*

Fructose concn. (M)	Sucrose (μmoles from 1 g fresh wt.)			
	Leaked		Stored	Net synthesis
	A	*B*		
0·1	20·0	3·4	39·1	62·5
0·2	27·0	11·1	40·2	78·3
0·3	23·0	17·0	38·1	78·1
0·5	20·7	25·5	32·0	78·2
0·7	23·3	34·5	21·3	79·3
0·9	16·5	43·7	15·5	75·5

* The slices (1 g fresh wt.) were incubated at 30° for 3 hr in 9·0 ml of fructose at the concentrations shown. Duplicate flasks were prepared for each fructose concentration. At the end of the 3-hr incubation period, the slices from one flask of each set were killed and extracted for the determination of tissue sucrose. The other flask of each set was used to determine sucrose leakage as follows: At the end of the 3-hr incubation period, 7·0 ml of the bathing solution were removed and analyzed for sucrose (*leakage A*). To the flasks containing 0·5, 0·7 and 0·9 M fructose a volume of water sufficient to reduce the fructose concentration of the remaining 2·0 ml of bathing solution to 0·1 M was rapidly added and removed prior to any further additions to the vessels. Then 8·0 ml of water were added to all flasks, each of which contained 2·0 ml of dilute fructose solution, and after an additional 1 hr of incubation the sucrose content of the bathing solution was determined (*leakage B*). Sucrose stored during the 3-hr incubation period was calculated using the equation given in the text.

taken up by scutellum slices at rates of 100–120 μmoles/hrg fresh wt. during the first 2 hr of incubation, thereafter the rate fell to 50–60 μmoles per hr. The lower rate provides the minimum hexose required to sustain a sucrose production of 25 μmoles per hr.

The effect of fructose concentration on the distribution of newly synthesized sucrose between the storage compartment and the compartment from which leakage can occur is shown in Table 2. The amount of sucrose stored during incubation in fructose was calculated by the equation:

$$S_s = S_t - (S_i + B)$$

where S_t = tissue sucrose after incubation in fructose, S_i = initial tissue sucrose before incubation (80–90 μmoles/g fresh wt) which is considered to be storage sucrose, B = sucrose *leakage B*, and S_s = sucrose stored during incubation.

Net sucrose synthesis was independent of fructose concentration in the range 0·2–0·9 M,

but was about 20 per cent lower in 0·1 M fructose. The major effect of fructose concentration was on the distribution of sucrose between storage and *leakage B* sucrose. As the fructose concentration of the bathing solution was increased there was an increase in the amount of *leakage B* sucrose and a decrease in the amount of sucrose stored. The fact that net sucrose synthesis is independent of fructose concentration while sucrose storage varies with fructose concentration indicates that there is no obligatory coupling between synthesis and storage. It appears from these results that high fructose concentrations (above 0·1 M) inhibit transport of newly synthesized sucrose which accumulates in an intracellular space from which it leaks (*leakage B*) upon dilution or removal of the fructose. During incubation in 0·1 M fructose very little sucrose accumulated in the *leakage B* compartment, and with the exception of *leakage A* (however, see below), sucrose synthesis and storage proceeded at the same rate.

Our interpretation of the data from the ^{14}C experiment (Table 1) was that *leakage A* sucrose resulted from the release of sucrose at the storage compartment membrane following an exchange between ^{14}C-fructose and the fructose moiety of the stored sucrose. If this is correct then *leakage A* represents a depletion of the storage sucrose pool during the 3-hr incubation period and includes not only some of the storage sucrose present at zero time but also some of the sucrose newly synthesized and stored during the incubation in fructose. That newly synthesized sucrose did occur in *leakage A* sucrose is indicated by the fact that the glucose moiety of *leakage A* sucrose was also labeled. This loss of storage sucrose should be taken into account in the calculation of S_s by adding an additional term (A, the amount of *leakage A* sucrose) to the right-hand side of the above equation. Thus, the storage sucrose values in Table 2 should be increased by the amounts of *leakage A*. The net sucrose synthesis values, however, would remain the same.

Inhibition of Sucrose Leakage B

The results presented in the previous section show that high concentrations of fructose inhibit sucrose *leakage B* since this leakage was observed only after dilution of the fructose in the bathing solution to 0·1 M or lower. Other sugars and polyhydric alcohols were also found to inhibit *leakage B* (Table 3). Inhibition greater than 90 per cent was found for all compounds

TABLE 3. INHIBITION OF SUCROSE *leakage B*

Additions to flask*	Final concn. (M)†	Sucrose leakage (μmoles/min)	Inhibition (%)
Water	—	1·86	—
D-Fructose	0·5	0·10	94·6
D-Galactose	0·4	0·09	95·2
D-Mannitol	0·4	0·08	95·7
myo-Inositol	0·4	0·05	97·3
D-Sorbitol	0·4	0·06	97·0
2,4-Dinitrophenol	5×10^{-4}	0·54	71·0
D-Fructose+DNP	$0{\cdot}5+5\times10^{-4}$	0·15	91·9

* The slices (1 g fresh wt.) were incubated at 30° for 3 hr in 9·0 ml of 0·5 M fructose. At the end of this period, 7·0 ml of the bathing solution were removed and 8·0 ml of water or the solutions shown above were added, after which sucrose leakage (*leakage B*) from the slices was followed with time.

† All flasks also contained 0·1 M fructose since 2·0 ml of the original 0·5 M fructose bathing solution remained in the flask and this was diluted to 10 ml upon addition of the solutions shown in the table.

tested with the exception of 2,4-dinitrophenol (DNP). Since the flasks contained 0·1 M fructose in addition to the inhibitor (see footnote, Table 3) sucrose *leakage A* was probably also occurring at a rate of about 0·1 μmole/min/g fresh wt. The leakage rates in the presence of sorbitol or inositol were well below this figure, and these compounds appear to cause some inhibition of sucrose *leakage A*, as well as an almost complete inhibition of *leakage B*. When mannitol (final concentration, 0·4 M) was added at the start of the 3-hr incubation in either 0·2 or 0·5 M fructose, the amount of *leakage A* was decreased about 40 per cent (8–9 μmoles). However, upon dilution of the bathing solution, the "missing" sucrose appeared along with *leakage B* sucrose.

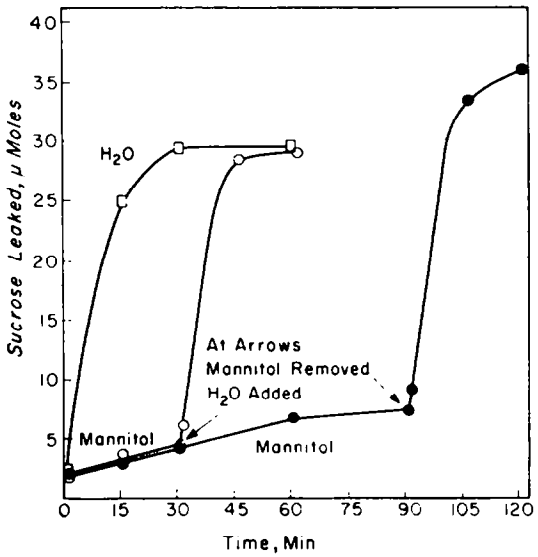

FIG. 4. MANNITOL INHIBITION OF SUCROSE *leakage B*.

The slices (1 g fresh wt.) were incubated at 30° for 3 hr in 0·5 M fructose. At the end of this period, 7·0 ml of the bathing solution were removed and 8·0 ml of either water or 0·5 M mannitol were added to the vessels. The final mannitol concentration was 0·4 M. Zero time in the graph is the time that the 8·0 ml additions to the flasks were made. Portions of the bathing solutions were removed for sucrose analysis at the times shown. At the time indicated by the arrows, 8·0 ml of the bathing solutions were removed and 8·0 ml of water were added to reduce the mannitol concentration to 0·08 M. The sucrose values shown have been corrected for the sucrose content of the 2·0 ml of original bathing solution which remained in the flask.

DNP inhibited the rate of *leakage B* by 71 per cent and after 90 min the amount of sucrose leaked into DNP was 80 per cent of the total leakage into the water control. DNP (5×10^{-4} M) also had a number of other effects on carbohydrate metabolism in the slices: (*a*) it caused a three-fold increase in the metabolic utilization of tissue sucrose (16–18 μmoles/hrg fresh wt.), (*b*) it inhibited hexose uptake about 80 per cent,[2] (*c*) in the presence of 0·2 M fructose and DNP there was a decrease (10 μmoles/hr) rather than an increase in tissue sucrose, (*d*) it caused a doubling of the intracellular hexose space of the slices.[2] This multiplicity of effects complicates the interpretation of the DNP inhibition of sucrose *leakage B*. However, DNP did not inhibit sucrose *leakage A*, this being additional evidence that the two types of sucrose leakage have different origins within the cell.

The effect of mannitol on the rate of *leakage B* is shown in Fig. 4. Almost complete inhibition of sucrose *leakage B* was obtained, and the sucrose was neither stored nor utilized

during the time it was prevented from leaving the tissue. The higher amount of *leakage B* sucrose appearing after 90 min in mannitol was undoubtedly due to sucrose synthesis since 0·1 M fructose was also present with the mannitol during the period of inhibition of leakage. Similar results were obtained with sorbitol and inositol. Mannitol did not inhibit metabolic utilization of stored sucrose since when freshly prepared slices were placed in 0·4 M mannitol or in water for 7 hr, the sucrose content of the slices in both flasks decreased to the same extent (5–6 μmoles/hr) and no net leakage occurred.

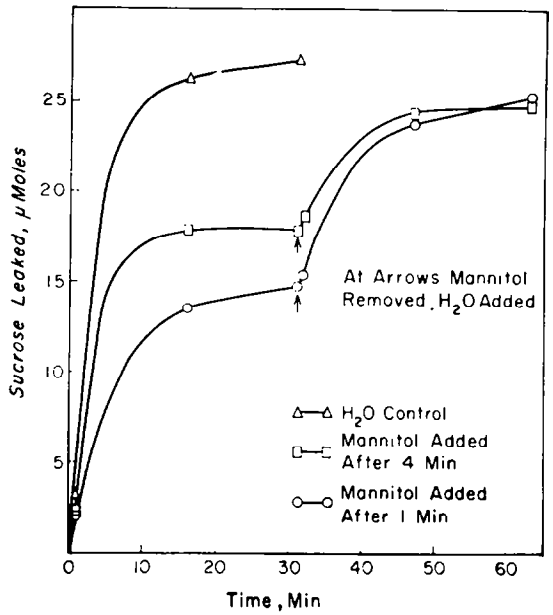

FIG. 5. EFFECT OF DELAYING MANNITOL ADDITION ON MANNITOL INHIBITION OF SUCROSE *leakage B*. The slices (1 g fresh wt.) were incubated at 30° for 3 hr in 9·0 ml of 0·5 M fructose. At the end of this period, 7·0 ml of the bathing solution were removed and 8·0 ml of water were added to the vessels. One min or 4 min after adding the water, 8·0 ml were removed from the flask and 8·0 ml of 0·5 M mannitol were added (final concentration of mannitol, 0·4 M). In the water control flask, 8·0 ml were removed after 4 min and 8·0 ml of water were added. Zero time in the graph is the time at which the final 8·0 ml addition was made. Some sucrose leakage occurred during the 1 or 4 min period after the first 8·0 ml addition: water control, 4 min, 0·5 μmole; mannitol, 1 min, 0·4 μmole; mannitol, 4 min, 2·9 μmoles. These amounts of sucrose are not included in the sucrose leakage shown above. After 31 min in mannitol all but 2·0 ml of the bathing solution were removed and 8·0 ml of water were added.

To obtain nearly complete mannitol inhibition of sucrose *leakage B*, the mannitol solution must be used to dilute the fructose solution. If the fructose solution was diluted with water and the mannitol added at short intervals following the initial dilution, part of the sucrose leakage *B* was not inhibited. These results are shown in Fig. 5. This experiment takes advantage of the initial lag in sucrose *leakage B* shown in Fig. 2, and the results indicate that, upon dilution of the fructose bathing solution with water, sucrose rapidly moves out of an intracellular space into a space from which its further movement is not inhibited by mannitol. The existence of an initial lag period in itself argues for another compartment but whether this latter compartment is intercellular or intracellular and identical to the hexose space of the scutellum[2] is not known.

DISCUSSION

On the basis of the results presented, it is postulated that the scutellum cells contain a sucrose storage space, presumably the vacuole, and a space within which sucrose is synthesized. The newly synthesized sucrose may be transported to the storage compartment or leaked to the cell exterior (*leakage B*). Movement to either place is inhibited by fructose; leakage to the cell exterior being the more strongly inhibited. The difference in the degree of inhibition, however, may have resulted from different fructose concentrations at the storage compartment membrane and the membrane at the cell exterior. From the results of the ^{14}C experiment (Table 1) and from the absence of a strong inhibition by fructose (Table 2) or DNP, it is postulated that *leakage A* sucrose comes from the storage compartment and moves to the cell exterior without passing through the compartment from which *leakage B* sucrose originates.

We have postulated that UDP-glucose-fructose glucosyltransferase is the enzyme-carrier involved in the transport and release of sucrose (*leakage A* sucrose) across the storage compartment membrane. It has been suggested that this enzyme is involved in sucrose breakdown; sucrose synthesis being catalyzed by UDP-glucose-fructose-6-phosphate glucosyltransferase.[4–8] Sucrose utilization (breakdown) by the scutellum slices occurred at a rate of about 5–6 μmoles/hr/g fresh wt. which was about the same rate as sucrose *leakage A* (Table 2, Fig. 3). The possibility exists, therefore, that in the absence of fructose the stored sucrose is transported across the storage compartment membrane and utilized in metabolism while in the presence of fructose the sucrose is released and appears as sucrose *leakage A*.

In the intact corn seedling, the sucrose of the scutellum is transported through the scutellar node to the developing root and shoot. Glucose, originating from the starch of the endosperm and acting in a manner similar to fructose, could give direction to this transport by inhibiting sucrose movement to the storage compartment and to the cell exterior on the endosperm side of the scutellum. Sucrose would thus move from areas of high glucose concentration to areas of low glucose concentration. Since the vascular strand runs longitudinally down the center of the scutellum, and since the scutellum is surrounded (except on the side containing the scutellar node) by endosperm tissue, it appears that the correct glucose gradient would be established. The glucose concentration of the endosperm tissue of 3–4-day old corn seedlings was found to be about 0·1 M. This concentration was calculated assuming equal distribution of the glucose throughout the water of the endosperm tissue, but higher concentrations may occur near the surface of the scutellum.

Our data on the inhibition of sucrose *leakage B* by fructose, galactose and polyhydric alcohols does not permit a definite conclusion as to whether or not this sucrose transport was carrier mediated. However, the fact that high concentrations (above 0·1 M) of inhibitor were necessary to inhibit leakage suggests that this transport does not involve a carrier system. Tukui and Hochster[9] showed that the rapid uptake of sucrose by *Agrobacterium tumefaciens* was inhibited in a competitive manner by a number of sugars including D-glucose, D-galactose and D-xylose. These sugars were strongly inhibitory at a concentration of 10^{-4} M when the sucrose concentration was 10^{-2} M, while their enantiomorphs were not inhibitory. Such results are best explained by assuming a carrier-mediated transport. On the other hand,

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inhibition by high concentrations of sugars or polyhydric alcohols may indicate a change in the structure of the membrane resulting in a decrease in permeability of the membrane to sucrose. Dainty and Ginzburg[10] found that the permeability of the tonoplast of *Nitella* to urea was markedly decreased by high concentrations of sucrose (0·5–1·0 molal), and suggested that this resulted from a shrinking of the membrane caused by a decrease in the chemical potential of the water in its neighbourhood.

EXPERIMENTAL

Plant Materials

Corn grains (*Zea mays* L., var. Funks G-76) were soaked in aerated 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 germinated 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

Each group of slices (1 g fresh wt.) was placed in a 25-ml Erlenmeyer flask containing. 9·0 ml of distilled water or fructose solution. The flasks were placed in a "Gyrotory" water bath at 30° (New Brunswick Scientific Company, New Brunswick, N.J.) and rotated at approximately 180 rev/min for a period which was usually 3 hr. After this period of incubation, 7·0 ml of the bathing solution (containing *leakage A* sucrose) were removed and additions of 8·0 ml of water or mannitol were made to each flask. One min after the addition to the vessels, two 0·2 ml samples were removed from the bathing solutions (containing *leakage B* sucrose), and the flasks were returned to the water bath. At predetermined intervals, additional duplicate 0·2 ml samples were removed from the vessels. These samples were used to determine sucrose *leakage B* into the bathing solution. In more complex experiments, the solutions bathing the tissue slices were removed and additions were made to the vessels more than a single time. The detailed procedures for these experiments are given in the Figures and Tables in the Results section of this paper.

In experiments requiring the incubation of tissue slices in ^{14}C-labeled fructose (Nuclear-Chicago Corp.) all vessels were equipped with center wells containing 0·2 ml of 10% KOH. The flasks contained 9·0 ml of 0·5 M [U-^{14}C] fructose (specific activity ~ 2860 counts/minμ-mole).

The tissue levels of sucrose at zero time and after various treatments were determined following the preparation of scutellum extracts. One gram of scutellum slices was placed in a 50-ml beaker and killed by the addition of 20 ml of boiling 80% ethanol, boiling was continued for 30 sec, and after 1 hr at room temperature the ethanol solution decanted into a 150-ml beaker. This was repeated and the slices were then washed with three 5-ml portions of 80% ethanol. The combined extracts were evaporated on a steam bath until the volume was reduced to approximately 2 ml. The remaining aqueous solution was transferred to a graduated 12 ml centrifuge tube, the pH adjusted to 7·0–7·2, and water added to bring the volume to 6·0 ml. The solution was centrifuged at 1000*g* for 10 min and the supernatant fluid decanted into a plastic tube and frozen. This solution was analyzed for its sucrose content.

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Carbohydrate Analysis

The sucrose contents of the bathing solutions 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 same method was used to determine the sucrose contents of the scutellum extracts; however, the tissue extracts were first cleared with $ZnSO_4$ and $Ba(OH)_2$ according to the method of Nelson.[11]

In the experiments involving the incubation of scutellum slices in ^{14}C-labeled fructose, the ^{14}C content of the leaked sucrose and the tissue sucrose as well as the ^{14}C distribution in the hexose moieties of the sucrose were determined following the separation of 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. Sucrose was located on the chromatograms by marker strips sprayed with aniline–diphenylamine and was quantitatively eluted from the paper with boiling 80% ethanol. Known quantities of bathing solution sucrose and tissue sucrose were then treated with invertase, and aliquots of the invert mixture were chromatographed on paper in the manner given above. The fructose and glucose areas of the chromatogram were located (with aniline–diphenylamine) and eluted with boiling 80% ethanol. The eluates were transferred to cupped, stainless steel planchets which were dried and then counted in a gas-flow counter.

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[11] N. Nelson, *J. Biol. Chem.* **153**, 375 (1944).