Phytochemistry, 1970, Vol. 9, pp. 1715 to 1723. Pergamon Press. Printed in England.

THE EFFECT OF URANYL NITRATE ON SUCROSE PRODUCTION AND STORAGE IN CORN SCUTELLUM SLICES*

T. E. HUMPHREYS and L. A. GARRARD

Department of Botany, Agricultural Experiment Station, University of Florida, Gainesville, Florida, U.S.A.

(*Received* 30 *October* 1969)

Abstract—Uranyl nitrate ($1{\cdot}5 \times 10^{-3}$ M) completely inhibited the storage of exogenous sucrose in slices of the corn scutellum, but had no effect on the storage of intracellularly synthesized sucrose. Sucrose production from glucose or fructose was inhibited by 15 and 35 per cent, respectively, and the inhibitions were not increased by a 10-fold greater uranyl ion concentration. Essentially the same degrees of inhibition were obtained when the slices were incubated with uranyl ion and sugar as when the slices were pretreated in uranyl ion and then washed and placed in sugar solutions. In the latter case, the inhibition was shown to be completely reversed by hydrogen ion or partially reversed by certain metal cations. Uranyl ion also strongly inhibited the exchange of sucrose between the bathing solution and the storage compartment of the cell. These results are consistent with a scheme which includes two sucrose transport membranes, one at the cell exterior for exogenous sucrose and the other at the cell interior between the sucrose synthesis and storage compartments and a membrane containing hydrophilic pores through which hexose molecules can freely diffuse from the cell exterior into the synthesis compartment.

INTRODUCTION

IN SLICES of the corn scutellum, exogenous sucrose is transported directly from the bathing solution into the storage compartment of the cell where it is accumulated.[1,2] Glucose and fructose are not accumulated by this tissue.[3,4] However, these hexoses enter a cellular compartment where sucrose is synthesized and the newly synthesized sucrose is then transported into storage.[5] We have concluded that both the storage of exogenous sucrose and the storage of newly synthesized sucrose require a membrane carrier. It appears, however, that there is a common storage pool although different storage mechanisms are involved.[1,5,6] In contrast, the hexoses freely diffuse into the cell, perhaps through hydrophilic pores in the plasmalemma.[4]

In this paper, we report studies on the effect of uranyl ion on sugar transport in slices of the corn scutellum. The effects of uranyl ion give additional support to a scheme where the storage mechanism for exogenous sucrose is at the cell surface and where the storage mechanism for newly synthesized sucrose is sequestered within the cell. These results are also consistent with the idea that hexoses enter the scutellum cell by free diffusion.

* Florida Agricultural Experiment Station Journal Series No. 3469. This investigation was supported, in part by a research grant (No. AM 07299-06) from the National Institute of Arthritis and Metabolic Diseases, U.S. Public Health Service.

[1] T. E. HUMPHREYS and L. A. GARRARD, *Phytochem.* **7**, 701 (1968).
[2] T. E. HUMPHREYS and L. A. GARRARD, *Phytochem.* **8**, 1055 (1969).
[3] T. E. HUMPHREYS and L. A. GARRARD, *Phytochem.* **3**, 647 (1964).
[4] L. A. GARRARD and T. E. HUMPHREYS, *Nature* **207**, 1095 (1965).
[5] T. E. HUMPHREYS and L. A. GARRARD, *Phytochem.* **5**, 653 (1966).
[6] L. A. GARRARD and T. E. HUMPHREYS, *Phytochem.* **8**, 1065 (1969).

RESULTS

During incubation of corn scutellum slices in fructose or glucose solutions, the hexose enters the synthesis compartment of the cell where sucrose synthesis occurs.[3-5] The newly synthesized sucrose may then be transported into the storage compartment. We consider sucrose to be stored if it does leak from the slices into water and, therefore, the term "storage compartment" has no special cytological meaning. As the concentration of exogenous hexose is increased above 0·1 M, there is a progressive inhibition of sucrose storage and some of the newly synthesized sucrose remains in the synthesis compartment where it can accumulate in appreciable amounts (e.g. 50 μmoles/g fr. wt.). When the hexose solution is replaced with water this accumulated sucrose leaks from the synthesis compartment. We refer to this sucrose as leakage B sucrose. During incubation in fructose, sucrose also leaks from the slices; however, this leakage originates in the storage compartment and appears to result from a different exit process. We refer to this sucrose as leakage A sucrose.[5]

To calculate the amount of sucrose produced and the amount of sucrose stored during incubation of scutellum slices in hexose solution, the amounts of leakage A and B sucrose and the change in the amount of tissue sucrose must be known. The amount of sucrose produced equals the sum of leakage A, leakage B and the change in tissue sucrose. The amount of sucrose stored equals the amount produced minus leakage B or the change in tissue sucrose plus leakage A.[5] The change in tissue sucrose is obtained by subtracting the amount of tissue sucrose before hexose incubation from the amount in the tissue after leakage B has occurred. Before the hexose incubation all the tissue sucrose is considered to be stored sucrose since it did not leak into water. When the slices are incubated in glucose, leakage A cannot be accurately determined (i.e. it is difficult to determine 15–20 μmoles of sucrose in the presence of 1000 μmoles of glucose). Therefore, the sucrose production values for glucose incubations are calculated assuming leakage A to be the same in glucose as in fructose. It is clear that fructose is the hexose of choice in these experiments. Assuming that the high respiratory demand for carbohydrate[7] is met by the abundant supply of fructose in the bathing solution, "true" values for sucrose production and storage can be obtained using fructose. However, when the scutellum slices are incubated in sucrose, only "net sucrose storage" values can be obtained (i.e. increase in tissue sucrose after incubation in sucrose) since neither the metabolic demand for sucrose nor the existence and magnitude of leakage A are known under these conditions.

The effect of uranyl ion on "net sucrose storage" from exogenous sucrose is shown in Fig. 1. Complete inhibition of storage was obtained at a uranyl ion concentration of 15×10^{-4} M. However, sucrose production from fructose or glucose was inhibited to a much less extent (Fig. 2). Since 0·1 M hexose concentrations were used in these experiments, leakage B was small (1–3 μmoles), and essentially all the sucrose synthesized in the presence of uranyl ion was stored. Maximum uranyl ion inhibitions of sucrose production from fructose (*ca.* 35 per cent) and from glucose (*ca.* 15 per cent) were obtained at a uranyl ion concentration of $1{\cdot}8 \times 10^{-3}$ M and the inhibition decreased at higher uranyl concentrations (up to 10-fold higher in the fructose-containing flasks). Uranyl salts undergo hydrolysis to give more complex uranyl ions and H^+. Thus, $1{\cdot}8 \times 10^{-3}$ M uranyl nitrate has a pH of 3·7 while 18×10^{-3} M uranyl nitrate has a pH of 3·1. The smaller inhibition of sucrose production at higher uranyl ion concentrations may have been due to this decrease in pH. When the slices were pretreated in uranyl nitrate solutions and then placed in fructose, sucrose production

[7] L. A. GARRARD and T. E. HUMPHREYS, *Phytochem.* 7, 1949 (1968).

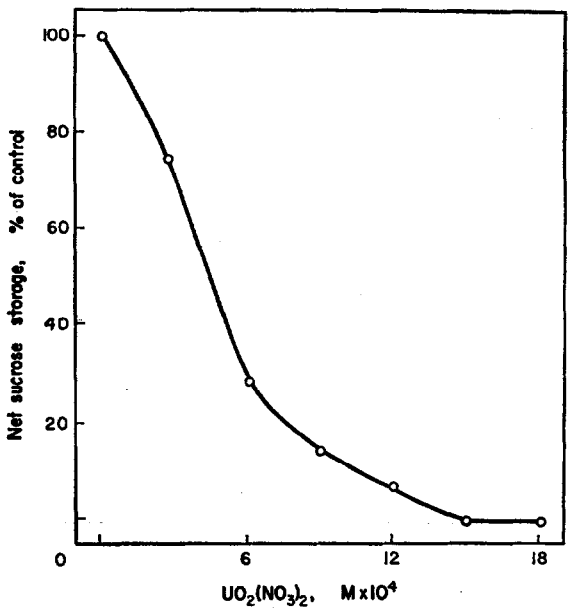

FIG. 1. THE EFFECT OF URANYL ION ON THE STORAGE OF EXOGENOUS SUCROSE BY SLICES OF THE CORN SCUTELLUM.

The slices (1·0 g fr. wt.) were incubated at 30° for 3 hr in solutions containing 0·1 M sucrose and the various concentrations of $UO_2(NO_3)_2$ shown above. The bathing solutions were then removed from the flasks by suction and the slices washed in 10 ml portions of water (×3). Then 10 ml of water were added to the flasks and the incubation continued for an additional 30 min in order to remove all leakable (unstored) sucrose. The tissue slices were then killed and extracted in hot ethanol and the sucrose contents of the alcohol extracts determined. The differences in the sucrose contents of freshly prepared, untreated tissue slices and of slices subjected to the above treatments were considered to be net sucrose storage values.

was also inhibited and these inhibitions were approximately the same as those obtained when uranyl ion and fructose were present together. However, as can be seen in Fig. 2, there was approximately a constant degree of inhibition over the uranyl concentration range $1{\cdot}8–18 \times 10^{-3}$ M. Leakage A was also inhibited by uranyl ion and to a greater extent than sucrose production. Note that although uranyl ion affected sucrose production, leakage A and exogenous sucrose storage to different degrees, the maximum effects were obtained in all cases at uranyl ion concentrations of the same order of magnitude ($1{\cdot}5–6 \times 10^{-3}$ M). We conclude from this that all the inhibitions result from uranyl ion binding to a single kind of ligand in the synthesis compartment and storage compartment membranes. These membranes are barriers between the synthesis compartment and the bathing solution and the storage compartment and the bathing solution and are thought to comprise different areas of plasmalemma.

Almost complete inhibition of exogenous sucrose storage was obtained when the slices were treated with uranyl nitrate solutions, washed and then placed in sucrose. However, when Al^{3+} was added to the sucrose solution only a small inhibition of sucrose storage was obtained. Mn^{2+} had a small effect in reversing the inhibition, but Ca^{2+}, Co^{2+} and Mg^{2+} were essentially inactive in this regard (Table 1). The Al^{3+} results may have been due, in part, to the low pH (Table 1); consequently, the effect of HCl on uranyl ion inhibition was tested.

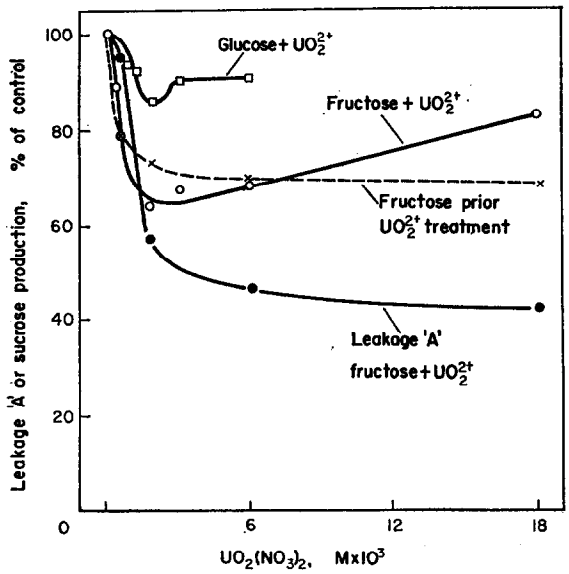

FIG. 2. THE EFFECT OF URANYL ION ON SUCROSE PRODUCTION FROM EXOGENOUS HEXOSE AND SUCROSE LEAKAGE A.

The slices (1·0 g fr. wt.) were incubated at 30° in solutions containing 0·1 M fructose or 0·1 glucose plus the various concentrations of $UO_2(NO_3)_2$ shown above. Fructose incubations were conducted for 3 hr while glucose incubations were conducted for 140 min. Following fructose incubations, samples of the bathing solutions were taken for the determination of sucrose leakage A (sucrose leakage A could not be determined when glucose was used as the exogenous hexose and it was assumed in calculating the glucose results that leakage A in glucose was the same as in fructose). At the conclusion of both fructose and glucose incubations, the slices were washed briefly in water and killed and extracted in hot ethanol for the determination of tissue sucrose (i.e. stored sucrose). In addition to the above treatments, tissue slices were incubated in water or in the various concentrations of $UO_2(NO_3)_2$ for 1 hr at 30° after which they were washed once in water and incubated subsequently for 3 hr at 30° in 0·1 M fructose. Samples of the bathing media were then taken for the determination of sucrose leakage A and the tissue slices were killed and extracted for the determination of tissue sucrose. The tissue content of sucrose was also determined for slices immediately after the pretreatment in water or in $UO_2(NO_3)_2$. The upper three curves represent sucrose production from hexose while the lower curve represents sucrose leakage during incubation in fructose (leakage A).

A 15-min wash in 0·01 M HCl following uranyl ion treatment completely abolished the inhibition of exogenous sucrose storage (Table 2). In a previous paper,[2] we showed that the inhibition of exogenous sucrose storage by treatment of scutellum slices in tris buffer (pH 7·5) was abolished in whole or part by Al^{3+}, Mn^{2+} and/or 0·01 M HCl. Presumably tris, a large monovalent cation and uranyl ion are reacting with the same ligand on the membrane surface.

The inhibition of sucrose production also was completely reversed by following uranyl ion treatment with a 15-min wash in HCl (Table 2). The inhibition of sucrose production from exogenous hexose also appears to be a result of the reaction of uranyl ion with the membrane surface.

In a previous paper,[1] we demonstrated that sucrose of the storage compartment exchanged with sucrose of the bathing solution. This exchange was not inhibited by citrate–phosphate buffers which completely abolished the storage of exogenous sucrose. In contrast to these results, treatment of the scutellum slices with uranyl ion strongly inhibited sucrose exchange (Fig. 3).

TABLE 1. REVERSAL OF URANYL ION INHIBITION OF EXOGENOUS SUCROSE STORAGE BY METAL CATIONS*

Prior treatment	Bathing solution (0·1 M sucrose + additions)	Net sucrose stored, μmoles	Initial pH of salt solution
H_2O	Alone	45·7	
$UO_2(NO_3)_2$	Alone	2·8	
$UO_2(NO_3)_2$	+$AlCl_3$ (0·02 M)	34·8	3·6
$UO_2(NO_3)_2$	+$MnCl_2$ (0·02 M)	11·2	5·2
$UO_2(NO_3)_2$	+$CaCl_2$ (0·02 M)	−5·5	6·0
$UO_2(NO_3)_2$	+$CoCl_2$ (0·02 M)	5·6	5·2
$UO_2(NO_3)_2$	+$MgCl_2$ (0·02 M)	2·8	5·3

* The slices (1·0 g fr. wt.) were incubated in water or $UO_2(NO_3)_2$ ($1·8 \times 10^{-3}$ M) at 30° for 1 hr after which they were washed twice with 10 ml of water and 10 ml of the above solutions were added to the flasks. Incubation of the slices in these solutions was continued for 3 hr at 30° after which the bathing media were removed and 10 ml of water were added to the flasks. The slices were incubated in water for 30 min to remove leakable sucrose (unstored sucrose) and then the tissue was killed and extracted in hot ethanol for the determination of tissue sucrose. In addition, groups of tissue slices were assayed for sucrose immediately following their prior treatment in water or $UO_2(NO_3)_2$ solution. The differences in the sucrose contents of the tissue slices following their prior treatment in water or $UO_2(NO_3)_2$ and after the final leakage period into water was considered to be net sucrose stored.

TABLE 2. REVERSAL BY HYDROGEN ION OF URANYL ION INHIBITIONS OF SUCROSE PRODUCTION AND STORAGE*

	Sucrose, μmoles					
Treatment sequence	Leakage A		Tissue change		Total stored	% Of control
H_2O, H_2O, sucrose			47·3			Control
UO_2^{2+}, H_2O, sucrose			1·4			3
UO_2^{2+}, HCl, sucrose			47·2			100
H_2O, H_2O, fructose	5·3	+	61·2	=	66·5	Control
UO_2^{2+}, H_2O, fructose	9·2	+	36·1	=	45·3	67
UO_2^{2+}, HCl, fructose	11·9	+	52·8	=	64·7	97

* Each group of slices (1·0 g fr. wt.) underwent three treatments in sequence. The first treatment was in water or $UO_2(NO_3)_2$ ($1·8 \times 10^{-3}$ M) and lasted for 1 hr. The second treatment was in water or HCl (10^{-2} M) and lasted for 15 min. The third treatment was in fructose (0·1 M) or sucrose (0·3 M) and lasted for 3 hr. All incubations were conducted at 30° and the slices were washed with 10 ml of water between each treatment. Three groups of slices were prepared for each treatment sequence. At the end of the second treatment in each sequence, one group of slices was killed, one group was placed in fructose and one group was placed in sucrose. Leakage A and change in tissue sucrose were determined as described in Figs. 1 and 2, footnotes.

Uranyl ion reacts with the storage membrane causing an inhibition of the storage of exogenous sucrose, an inhibition of leakage A and an inhibition of sucrose exchange. Inasmuch as sucrose has been shown to move across the synthesis membrane (leakage B),[2,6] experiments were conducted to test the effect of uranyl ion on the ability of the tissue to

accumulate sucrose within the synthesis compartment and on the rate at which the accumulated sucrose leaks into the bathing solution (leakage B). After treatment with uranyl ion, the slices retained their ability to accumulate sucrose in the synthesis compartment (Table 3). The smaller amount of leakage B sucrose obtained from slices treated with uranyl ion was undoubtedly a result of the smaller amount of sucrose production since both leakage B and

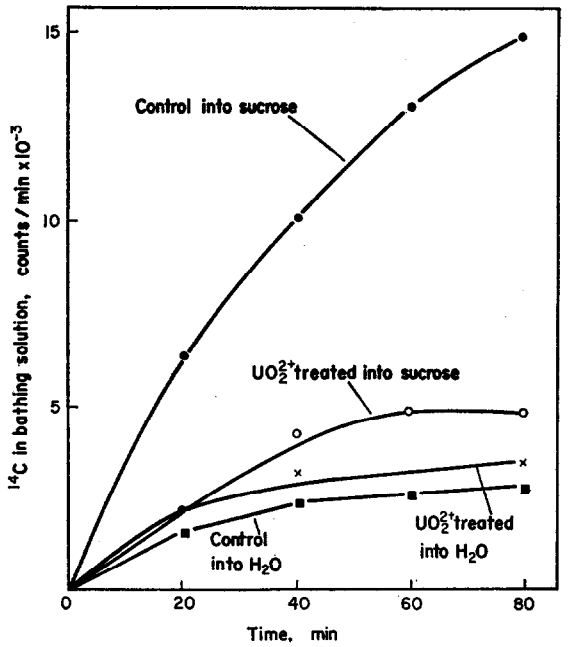

FIG. 3. THE EFFECT OF URANYL ION ON SUCROSE EXCHANGE.

The slices (1·0 g fr. wt.) were placed in closed flasks which contained $1·1 \times 10^{-2}$ 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 with 10 ml water (×3) and either 9·0 ml of water or $1·8 \times 10^{-3}$ M $UO_2(NO_3)_2$ were added to the flask. After an additional 60 min incubation in either water or $UO_2(NO_3)_2$ the slices were washed once in 10 ml of water and groups from each treatment were incubated either in water or 0·05 M sucrose for 100 min with samples being taken periodically from the bathing solutions to determine the release of ^{14}C from the tissue. Samples were plated in stainless-steel planchets, dried and counted in a gas-flow counter. Corrections were made for background and self-absorption.

sucrose production were inhibited to approximately the same degree. It is clear, moreover, that treatment with uranyl ion did not prevent the movement of sucrose through the synthesis membrane into the bathing medium. When slices which had been incubated in 1·0 M fructose in order to load the synthesis compartment with sucrose were placed in uranyl nitrate solutions instead of water, leakage B was inhibited (Fig. 4). Considering the amount of sucrose leaked after 15 min, inhibitions of 18 per cent and 39 per cent were obtained at uranyl nitrate concentrations of $1·8 \times 10^{-3}$ M and 18×10^{-3} M, respectively. These inhibitions were less than those obtained with Al^{3+} or Ca^{2+} but greater than that obtained with Mg^{2+} at similar concentrations.[2,8]

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

TABLE 3. EFFECT OF PRIOR TREATMENT OF THE SLICES WITH $UO_2(NO_3)_2$ ON THE LEAKAGE OF SUCROSE FROM THE SYNTHESIS COMPARTMENTS OF THE CELLS (LEAKAGE B)*

Prior treatment	Leakage B sucrose, μmoles	% Of control	Total sucrose production, μmoles	% Of control
H_2O	23·2	Control	104·3	Control
$UO_2(NO_3)_2$	16·2	70	77·1	74

* The slices (0·1 g fr. wt.) were placed in $UO_2(NO_3)_2$ ($1{\cdot}8 \times 10^{-3}$ M) or in water for 1 hr at 30°. The flasks were run in duplicate and at the end of 1 hr treatment, the slices from one flask of each set were killed and extracted (the sucrose contents of the slices after H_2O treatment were 52·8 μmoles/g and after uranyl ion treatment were 56·9 μmoles/g). The slices in the second flask of each set were washed twice with 10 ml portions of water and then incubated in 0·5 M fructose for 3 hr at 30°. At the end of this period, the bathing solution was removed, the slices were quickly washed with 10 ml of water and 10 ml of water were added to the flask. The slices were incubated at 30° for an additional 45 min and then a portion of the bathing solution was removed for sucrose analysis (leakage B). The tissue slices were killed and extracted in order to determine tissue sucrose.

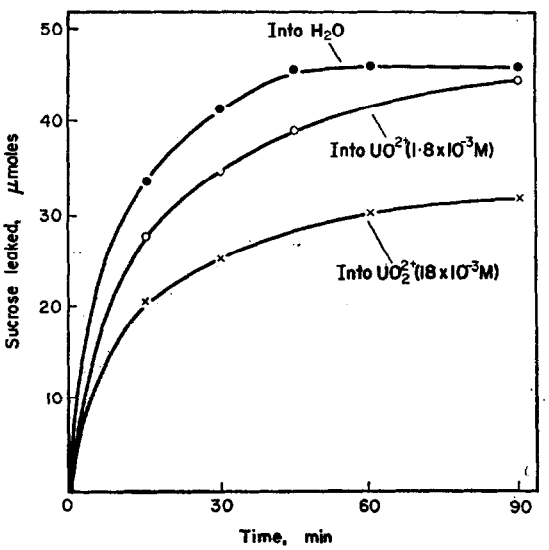

FIG. 4. THE INHIBITION OF SUCROSE LEAKAGE B BY URANYL ION.

The slices (1·0 g fr. wt.) were incubated in 1·0 M fructose at 30° for 3 hr. At the end of this period, the bathing solution was removed from the flask by suction, and the slices were washed by the rapid addition and removal of 10 ml of water or $UO_2(NO_3)_2$ ($1{\cdot}8 \times 10^{-3}$ M and 18×10^{-3} M). Then 10 ml of water or $UO_2(NO_3)_2$ solutions were added to the flasks (time zero on the graph). Portions of the bathing solution were removed for sucrose analysis at the times shown.

DISCUSSION

Uranyl ions inhibit sugar uptake in mammalian intestine[9,10] and yeast.[11] In yeast the uptake of glucose (active uptake) is very sensitive to uranyl ion while the uptake of sorbose

[9] F. PONZ and M. LLUCH, *Rev. Esp. Fisiol.* **14**, 217 (1958).
[10] H. NEWEY, P. A. SANFORD and D. H. SMYTH, *J. Physiol.* **186**, 493 (1966).
[11] A. ROTHSTEIN and J. VAN STEVENINCK, *Ann. N.Y. Acad. Sci.* **137**, 606 (1966).

(facilitated diffusion) is less sensitive.[12] However, at the proper uranyl ion concentration complete inhibition of uptake of both sugars is obtained. With sorbose it was shown that not only the influx but also the efflux was inhibited by uranyl ion.[13] Rothstein and Van Steveninck[11] suggest that these inhibitions result from uranyl ion binding to phosphoryl groups in the membrane (when active uptake is inhibited) or to carboxyl groups in the membrane (when facilitated diffusion is inhibited). Newey *et al.*[10] found that while 3×10^{-4} M uranyl nitrate substantially reduced mucosal transfer of glucose in rat intestine, it had no effect on the transfer of 3-*O*-methylglucose or galactose. A uranyl ion concentration of 3×10^{-3} M was required to inhibit the transfer of the latter two sugars. Since all these sugars are actively absorbed by the intestine,[14] it is not possible in this tissue to distinguish between active uptake and facilitated diffusion on the basis of uranyl ion sensitivity alone.

In the corn scutellum, the storage of exogenous sucrose was completely inhibited by uranyl ion while the storage of sucrose from the synthesis compartment was unaffected. Both of these storage processes can move sucrose against a concentration gradient and both, presumably, involve membrane carriers.[1,6,8] The transport of sucrose from the synthesis compartment into storage occurs at an intracellular location while the transport of exogenous sucrose into storage occurs at or near the cell exterior where the uranyl ion can penetrate. Clearly, the fact that the uranyl ion inhibitions of exogenous sucrose storage and sucrose production were reversed by HCl indicates that the action of the uranyl ion is limited to the cell surface.

The inhibition of sucrose production by uranyl ion does not appear to involve hexose transport into the synthesis compartment since about the same degree of inhibition was obtained at 0·1 and 0·5 M fructose concentrations (Fig. 2, Table 3) and since the inhibition did not increase when the uranyl ion concentration was increased 10-fold (Fig. 2). A more likely explanation is that some molecules of an enzyme whose activity limits the rate of sucrose production (e.g. hexokinase) are in the cell surface and are inhibited by uranyl ion. The majority of the enzyme molecules, however, are within the cell boundary and are protected from uranyl inhibition.

Glucose, fructose and mannose rapidly equilibrate between the synthesis compartment of the scutellum cell and the bathing solution.[4] Furthermore, entrance of glucose into the synthesis compartment (hexose space) is not inhibited by fructose, mannose, methyl-*D*-glucose, glucosamine, *N*-acetylglucosamine or mannitol.[4] We have concluded, therefore, that hexoses enter the synthesis compartment by free diffusion through hydrophilic pores in the membrane. The apparent lack of inhibition of hexose transport by uranyl ion is consistent with this conclusion. The transport of fructose in the rat intestine[10] is also insensitive to uranyl ion (3×10^{-3} M). Fructose is passively absorbed by the intestine[14] but it is not known whether this involves free diffusion or carrier-mediated transport (facilitated diffusion).

Leakage A is inhibited not only by uranyl ion (Fig. 2) but also by Ca^{2+} and Mn^{2+}.[8] These latter ions, however, do not inhibit the storage of exogenous sucrose even at concentrations as high as 0·02 M (unpublished results). Perhaps uranyl ion cannot be displaced by sucrose from its attachment to the membrane, while Ca^{2+} and Mn^{2+} are displaced. In this regard, glucose displaces Co^{2+} from the cell surface of yeast but not uranyl ion.[12,15] During leakage A, however, the sucrose is separated by the storage membrane from the cation solution bathing the cell and therefore cannot displace the cation from the outer membrane surface. We

[12] J. VAN STEVENINCK and A. ROTHSTEIN, *J. Gen. Physiol.* **49**, 235 (1965).
[13] V. P. CIRILLO, *Trans. N.Y. Acad. Sci.* **23**, 725 (1961).
[14] R. K. CRANE, *Physiol. Rev.* **40**, 789 (1960).
[15] J. VAN STEVENINCK and H. L. BOOIJ, *J. Gen. Physiol.* **48**, 43 (1964).

have no evidence that leakage A and the storage of exogenous sucrose involve the same transport system. Leakage A, however, does not occur by simple diffusion.[5] It is also not clear whether sucrose exchange across the storage membrane is an aspect of the sucrose storage mechanism or the leakage A mechanism or both. In any case, movement of sucrose in either direction across the membrane separating the storage compartment and the outside solution is quite sensitive to uranyl ion. These sucrose movements appear to be carrier mediated. Movement of sucrose from the synthesis compartment into the bathing solution (leakage B) is less sensitive to uranyl ion, and movement of hexose from the cell exterior into the synthesis compartment appears to be insensitive to uranyl ion. These sugar movements are thought to occur by free diffusion through hydrophilic pores in the membrane.[2,4,8]

EXPERIMENTAL

Plant Materials

Corn grains (*Zea mays* cv. 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 1·0 g.

Experimental Procedure

While the detailed procedures for these experiments are given in the Tables and Figures, certain methods remained the same throughout these investigations. Each group of slices (1·0 g fr. wt.) was placed in a 25 ml Erlenmeyer flask containing 10·0 ml of the appropriate bathing solution. Incubation of the slices was conducted at 30° in a "Gyrotory" water bath (New Brunswick Scientific Company, New Brunswick, N.J.). The handling of samples taken from the bathing solutions for the determination of sucrose leakage A and leakage B and the preparation of the ethanolic extracts of the tissue slices have been described previously.[8]

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.

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