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SUGAR UPTAKE IN THE MAIZE SCUTELLUM*

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Key Word Index—*Zea mays*; Gramineae; maize scutellum; sucrose and glucose uptake; active transport; diffusion.

Abstract—Characteristics of the uptake of sucrose, glucose and fructose by maize scutellum slices are presented. Sugars were taken up at almost a constant rate until the bathing solution was nearly depleted, even when initial sugar concentrations were well below those which saturated the uptake mechanisms. DNP, phloridzin, uranyl ion, and anoxia were more inhibitory to the uptake of sucrose than to the uptake of hexoses. Maltose was taken up without hydrolysis. Turanose was not taken up, but it slightly inhibited the uptake of sucrose. The uptake of sucrose, glucose and fructose was accompanied by aerobic fermentation. The following conclusions are drawn; (a) sucrose is taken up actively without inversion; (b) hexoses are taken up by two processes, diffusion and active transport operating simultaneously; (c) the active uptake mechanisms for both sucrose and the hexoses are coupled to glycolysis.

INTRODUCTION

SLICES of the corn scutellum take up glucose and fructose at rapid rates and there is a concomitant synthesis and storage of sucrose.[1,2] Glucose and fructose are not accumulated.[1,3] The sucrose synthesis compartment is free space to hexoses which, however, cannot penetrate the sucrose storage compartment.[3] Hexose uptake is accompanied by a rapid rate of aerobic, ethanolic fermentation.[4] Sucrose is taken up without hydrolysis by a mechanism located at the plasmalemma.[5,6]

Results presented in this paper indicate that glucose is taken up by an active process operating simultaneously with simple diffusion. Additional evidence is presented to show that sucrose is taken up actively without inversion. Data are consistent with a scheme whereby the active uptake of both sucrose and the hexoses are coupled to a specific glycolytic step.

RESULTS

Characteristics of Sucrose Uptake

Slices of the corn scutellum take up sucrose from a 1 to 7·5 mM solution at almost a constant rate over a period of 2 hr in spite of the fact that the concentration of the bathing solution is considerably reduced as a result of uptake. At 7·5 mM initial concentration of

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[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, *Nature, Lond.* **207**, 1095 (1965).
[4] L. A. GARRARD and T. E. HUMPHREYS, *Phytochem.* **7**, 1049 (1968).
[5] T. E. HUMPHREYS and L. A. GARRARD, *Phytochem.* **7**, 701 (1968).
[6] T. E. HUMPHREYS and L. A. GARRARD, *Phytochem.* **9**, 1715 (1970).

sucrose in the bathing solution, the concentration after 90 min was 70% of that at the beginning. At 1 mM initial concentration of sucrose, the amount was reduced by 60% during the 2-hr incubation. The rate of uptake increased after the first 30-min measurement period. In subsequent experiments the first sample was taken 15 min after adding the sucrose solution.

The effect of concentration of sucrose on the rate of uptake is shown in Fig. 1. In these experiments, sucrose uptake was measured over a period of 1 hr with the three lowest concentrations and over a period of 3 hr with the two highest concentrations. Sucrose uptake proceeded at a constant rate over the period during which uptake was measured. As shown by the theoretical curve (Fig. 1), the data agree with the typical Michaelis and Menten hyperbolic substrate concentration curve for which the constants were derived from a Lineweaver–Burk plot of the data.

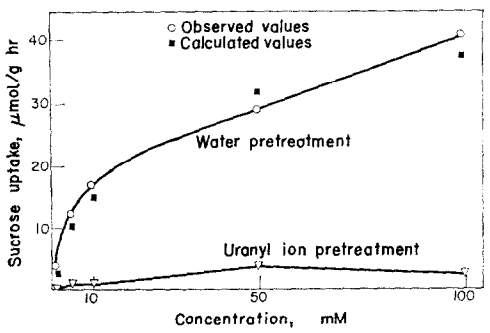

FIG. 1.

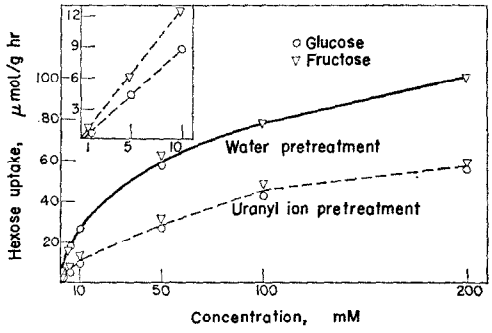

FIG. 2.

FIG. 1. THE EFFECT OF WATER OR URANYL ION PRETREATMENT ON SUCROSE UPTAKE.
Slices (1·0 g fr. wt) were placed in either water or 3 mM uranyl nitrate for 1 hr. The slices were rinsed twice, placed in water for 30 min, rinsed twice more and finally placed in the bathing solutions. Two samples were taken, the first 15 min after the bathing solution was added and the second at the end of the uptake period. Uptake was measured over periods of 60 min with 1 mM and 5 mM sucrose, 90 min with 10 mM sucrose and 180 min with 50 mM and 100 mM sucrose. (Constants for V_{max} and K_m of 45 μmol/g hr and 20 mM were used to obtain the calculated curve.)

FIG. 2. THE EFFECT OF URANYL ION PRETREATMENT ON HEXOSE UPTAKE.
Conditions as in Fig. 1.

Sucrose uptake increased with time when the bathing solution was maintained at a constant sucrose concentration (1 mM). In this experiment, the volume of the bathing solution was reduced to 4 ml for greater accuracy. At the end of each 15-min uptake period the solution was removed and fresh 1 mM sucrose was added. The rate of uptake increased with time over a period of 2 hr.

The effect of tissue sucrose level on the rate of sucrose uptake is shown in Table 1. The tissue was subjected to various treatment sequences to vary the amount of sucrose in the tissue prior to measuring uptake. Generally, there was an approximate inverse correlation between the amount of sucrose in the tissue and the rate of uptake.

The amount of inversion of exogenous sucrose was small as measured by the appearance of glucose in the bathing solution. For instance, at a sucrose concentration of 50 mM the maximum amount of glucose noted in the solution was 0·6 mM. This concentration of glucose is not sufficient to account for the observed rate of sucrose uptake. It is assumed, of course, that the inverted sugars are free to diffuse into the surrounding medium. Uranyl ion

TABLE 1. THE EFFECT OF TISSUE SUCROSE LEVEL ON THE RATE OF SUCROSE UPTAKE*

Treatment sequence	Sucrose in tissue (μmol/g)	Sucrose uptake (μmol/g hr)
Water 3 hr, rinse, water 1 hr, rinse, sucrose solution	50	25
Water 1 hr, rinse, sucrose solution	68	17
20 mM fructose 3 hr, rinse, water 1 hr, rinse, sucrose solution	81	21
100 mM fructose 3 hr, rinse, water 3 hr, rinse, sucrose solution	94	18
100 mM fructose 3 hr, rinse, water 1 hr, rinse, sucrose solution	125	15
100 mM fructose 3 hr, rinse, sucrose solution	133	11

* The results from three crops of seedlings are reported here. In each case the uptake period was 1 hr, the sucrose from which uptake was measured was 10 mM and there was a 15-min delay between adding the uptake solution and taking the first sample. Duplicate samples were subjected to the sequences as listed, and, at the beginning of the uptake period, one group was killed for sucrose analysis while the other was used to measure sugar uptake.

inhibited the uptake of sucrose in excess of 90% whereas hexose uptake was inhibited by about 50% (see Figs. 1 and 2). If sucrose were being inverted *prior* to uptake, uranyl ion should only halve the uptake of sucrose. It might be argued that uranyl ion inhibits the inversion of sucrose; however, the amount of glucose found in the bathing solution is higher after uranyl ion pretreatment than it is after water pretreatment. These results suggest, in agreement with an earlier report,[5] that sucrose is taken up without inversion.

When maltose, an expected product of the breakdown of starch in the maize endosperm, was supplied to the scutellum slices there was considerable hydrolysis; and the glucose concentration of the bathing solution increased throughout the uptake period whereas the concentration of maltose declined (Table 2). Uptake is expressed in terms of μmol of glucose. The concentrations of glucose shown in Table 2 are not sufficient to account for the rates of sugar uptake (Fig. 2) indicating that some maltose was taken up without hydrolysis. Indeed, in the case of 50 mM maltose, it appears that most of the sugar was taken up as maltose since the maximum observed concentration of glucose (8·6 mM Table 2) would only support the uptake (Fig. 2) of 25 μmol of glucose leaving 64 μmoles of glucose to be taken up as maltose (32 μmol maltose). This is the rate at which sucrose is taken up (Fig. 1).

Characteristics of Hexose Uptake

As with sucrose, the initial rates of hexose uptake could be maintained until the bathing solution was nearly depleted. This was verified in numerous experiments with several concentrations of hexose (Fig. 3). The data from experiments in which the course of uptake of sucrose and the hexoses was followed with time are both therefore contrary to what would be predicted by Michaelis and Menten kinetics. Furthermore, the curve obtained when the

TABLE 2. MALTOSE UPTAKE BY SCUTELLUM SLICES*

Concn of added maltose (mM)	Glucose (μmol/flask) initial	final	Disappearance of maltose (μmol/flask)	Sugar uptake (as μmol glucose/flask)
5	7	26	18	17
10	11	42	30	29
50	23	86	76	89

* Slices (1·0 g fr. wt) were incubated in water for 1 hr, rinsed twice, and then placed in the various concentrations of maltose; 15 min after adding the maltose the first 2 ml sample was taken. The second sample was taken one hr after the first. Glucose was measured by using 'Glucostat'. Reducing sugars were run on the same solutions and maltose determined by difference. Reducing sugar standard curves were obtained for both sugars.

rate of hexose uptake is plotted as a function of hexose concentration (Fig. 2) is not a rectangular hyperbola or a diffusion curve.

However, when the slices were pretreated with uranyl ion, hexose uptake appeared to follow diffusion kinetics. The dotted lines in Fig. 2 show the uptake vs. concentration data for glucose and fructose when uptake was measured following a pretreatment with uranyl ion. Since glucose is not accumulated by the tissue but is rapidly used for sucrose synthesis or is respired and fermented, the question arises as to whether the kinetics presented are those of glucose diffusion into the tissue or glucose utilization (hexokinase reaction) within the tissue. Jones[7] studied the properties of hexokinase from the corn scutellum and reported the K_m for glucose as $6{\cdot}5 \times 10^{-6}$ M. It appears from this low K_m that diffusion and not the hexokinase reaction is limiting uptake. If diffusion is the process by which uptake occurs and if it is assumed that the internal glucose concentration is low and constant, then the uptake should be a straight line function of the glucose concentration. A straight line, in fact, was obtained at concentrations below 10 mM (inset Fig. 2). The deviation from linearity at concentrations above 10 mM may have been due to higher internal concentrations of glucose and to a saturation of the glucose utilization process at the higher concentrations.

The idea that hexose uptake takes place, in part, by diffusion is supported by experiments on the time course of glucose uptake into uranyl nitrate-treated and untreated slices (Fig. 3). The glucose uptake after uranyl nitrate pretreatment is typical of a diffusion curve. If an arbitrary constant is multiplied by the concentration at the beginning of each period of uptake, the curve represented by the dotted line is obtained. In contrast, when untreated slices were used, uptake proceeded at almost a constant rate until the glucose in the bathing solution was depleted to a level below that of the detection system used for glucose analysis (Fig. 3). This was true despite the fact that the tissue is capable of taking up glucose at much higher rates (Fig. 2).

It is postulated, therefore, that hexose uptake is the total of two processes: one, consisting of simple diffusion, is seen after uranyl ion treatment; and one, an active process, is subject to inhibition by uranyl ion binding.

When the glucose concentration in the bathing solution was kept constant the rate of uptake was also constant, and the uptake over a period of 2 hr was essentially that obtained

[7] H. C. JONES, Ph.D. Thesis, University of Florida, Gainesville (1965).

with declining glucose concentration. This was in contrast to results obtained with sucrose uptake where the rate increased with time.

Inhibitors of Sugar Uptake

The effects of anoxia on the uptake of sucrose, glucose and fructose were determined. After a 1-hr water incubation at room temperature the slices were rinsed, 10 mM sugar solution was added, and flasks were either agitated in air or placed under nitrogen which was continuously bubbled through the bathing solution. Sucrose uptake in nitrogen was 33% of that in air whereas with glucose and fructose the rates of uptake in nitrogen were 65% of those in air.

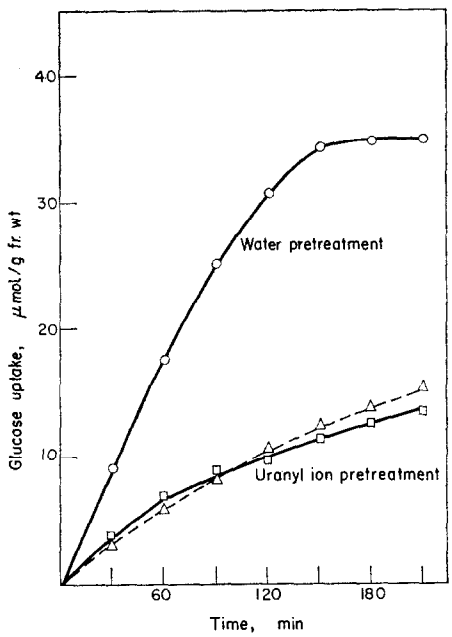

FIG. 3.

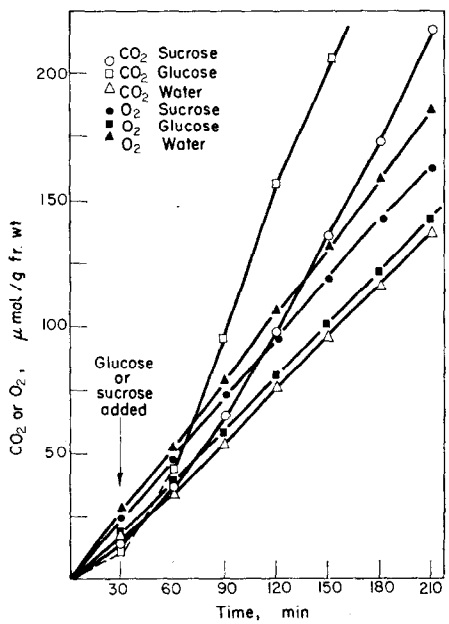

FIG. 4.

FIG. 3. URANYL ION PRETREATMENT AND GLUCOSE UPTAKE.
The pretreatment consisted of 1 hr in either water or 3 mM uranyl nitrate. The slices (1·0 g fr. wt) were then rinsed twice, left for 30 min in water, rinsed twice more and then 10 mM glucose solution was added. The first sample was taken 8 min after the solution was added and samples were taken every 30 min thereafter for a period of 210 min. The volume of the uptake solution was 6 ml (see text for explanation of dotted line).

FIG. 4. GAS EXCHANGE IN WATER AND 100 mM SUCROSE OR GLUCOSE.
Experiments were r un with 100 mg tissue per flask. The sugar solution (1·0 M) was added from the side arm 30 min after readings were begun.

The inhibition of sugar uptake by phloridzin is shown in Table 3. At 1 mM, phloridzin inhibited sucrose uptake about twice as much as glucose uptake; however, the uptake of neither sugar was inhibited strongly by the low concentrations used with animal systems. Dinitrophenol (DNP) also inhibited sugar uptake (Table 3). The inhibition was considerably greater with sucrose than with glucose; the sucrose inhibition approached 100% whereas the inhibition of glucose uptake approached 50%.

TABLE 3. INHIBITION OF SUGAR UPTAKE BY PHLORIDZIN AND DNP*

Concn of inhibitor M ($\times 10^{-4}$)	Inhibition (%) Glucose	Sucrose	Concn of inhibitor M ($\times 10^{-4}$)	Inhibition (%) Glucose	Sucrose
Phloridzin			DNP		
0·1	0	—	0·1	2	31
1	11	0	0·3	14	51
5	—	14	1	35	89
10	13	25	3	43	94
20	—	47			
30	—	60			

* The slices (1·0 g fr. wt) were incubated in water for 1 hr, rinsed twice, and placed in a solution of 0·01 M sugar and inhibitor as indicated. Results are based on a non-inhibited control. Uptake was measured over 2 hr (phloridzin) and 90 min (DNP).

The uptake of sucrose was measured in the presence of several disaccharides. Slices were incubated for 1 hr in water and then placed in 10 mM sucrose plus the various disaccharides at concentrations of 5–50 mM, for an uptake period of 1 hr. Sucrose uptake was not inhibited by melibiose, trehalose or cellobiose. With lactose and sucrose together there was more hydrolysis, as measured by the appearance of glucose in solution, than with either sugar alone. If this glucose came from the hydrolysis of sucrose, then the uptake of sucrose was inhibited about 30% by 50 mM lactose. Turanose at 50 mM caused approximately a 30% inhibition of sucrose uptake (Table 4). When turanose and sucrose were added to the slices, the glucose readings were little or no higher than when sucrose was added alone. That the effect of turanose was not osmotic was demonstrated by measuring the uptake of sucrose alone, sucrose in the presence of turanose and sucrose in the presence of mannitol. When turanose alone was added to the slices no glucose appeared in solution. Neither lactose nor turanose was taken up by the slices.

TABLE 4. THE EFFECTS OF TURANOSE AND MANNITOL ON SUCROSE UPTAKE*

Experiment	Sucrose uptake (μmol/g hr) 10 mM sucrose	10 mM sucrose + 50 mM turanose	10 mM sucrose + 50 mM mannitol
1	15·2	10·1	
2	15·5	11·7	
	16·0	12·2	
	16·5	12·7	
3	16·1	11·4	15·6
	17·1	14·6	16·6

* Slices (1·0 g fr. wt) were incubated for 1 hr in water, rinsed twice, and the indicated solutions were added. The first sample was taken 15 min after addition of the uptake solution. Uptake was measured for 1 hr in Experiment 1 and 2 hr in Experiments 2 and 3.

Sugar Induced Fermentation

Figure 4 shows the gas exchange in 10 mM sucrose compared to that in water. Slices in water had an RQ of less than 1·0 and both O_2 uptake and CO_2 evolution proceeded at constant rates throughout the experiment. When sucrose was added there was a very slight

depression in the rate of O_2 consumption; the rate of CO_2 evolution, on the other hand, slowly increased throughout the experiment. When glucose was added to slices in water, the rate of O_2 consumption remained nearly constant whereas the evolution of CO_2 greatly increased (Fig. 4). The *RQ* during the last period measured was 2·3. Over the period of these experiments, the uptake of both sucrose and glucose proceeded at a constant rate.

DISCUSSION

The following conclusions are drawn: (a) sucrose is taken up actively without inversion; (b) hexoses are taken up by two processes, diffusion and active transport, operating simultaneously; and (c) the active uptake mechanisms for both sucrose and the hexoses are coupled to glycolysis.

That sucrose is taken up without hydrolysis is supported by the following results: the small amount of extracellular inversion of sucrose; the differences in the kinetics of sucrose and hexose uptake; the different effects of uranyl ion on sucrose and hexose uptake. The data of Table 2 show that maltose also is taken up by the tissue without hydrolysis and at rates similar to those for sucrose.

That sucrose uptake is an active process is indicated by the fact that it was taken up against concentration gradients. Slices that contained 133 μmol sucrose/g (130 mM sucrose) took up sucrose from a 10 mM solution (Table 1). Slices incubated in water for 1 hr contained 68 μmol sucrose/g (68 mM sucrose) and took up sucrose from a 1 mM solution. The sucrose concentrations given above are minimum values since an equal distribution of sucrose throughout the tissue water was assumed. The inhibition of sucrose uptake by DNP is consistent with the idea of a metabolic energy-requiring process. Inhibition of sucrose uptake by turanose (and probably by lactose) fulfills one of the criteria (competition by similar compounds) for a facilitated diffusion or an active transport process.[8]

The scutellum also appears to have an active hexose transport mechanism. The constant rate of glucose uptake until the bathing solution was nearly depleted and the hexose uptake vs. concentration curves (Fig. 2) can both be explained by a combination of diffusion and active transport. The diffusive component of uptake is indicated by the time–course curve of glucose uptake after uranyl ion treatment (Fig. 3) which is a typical diffusion curve. This curve could also be the result of an enzymatic process with a very high K_m (above 50 mM). The uptake rate vs. concentration curves for the hexoses (Fig. 2) do not resemble rectangular hyperbolas nor do they represent the kinetics of a diffusion process except after treatment with uranyl ion in which case, at lower hexose concentrations, diffusion kinetics are represented (i.e. a straight line is obtained). Since glucose is not accumulated by the scutellum cells but is used to form sucrose or is catabolized, glucose which diffuses into the tissue is assumed to be phosphorylated by a cytoplasmic hexokinase whereas glucose taken up actively is phosphorylated perhaps at the plasmalemma. This is active transport in the sense that uptake is coupled to another reaction at the membrane, but it is not active transport in the sense that glucose is being accumulated against a gradient. The active process described here is termed 'group translocation' by Roseman.[9]

The idea that sucrose uptake is active whereas glucose uptake is in part active and in part passive is supported by the inhibitor studies. Phloridzin, DNP, and anoxia inhibited the uptake of sucrose to a greater extent than the uptake of glucose. This effect would be expected if the active and not the passive portion of glucose uptake were being inhibited.

[8] W. D. Stein, *The Movement of Molecules across Cell Membranes*, Academic Press, New York (1967).
[9] S. Roseman, *J. Gen. Physiol.* **54**, 138 (1968).

A combination of active transport and facilitated diffusion is thought to be involved in the uptake of glucose by yeast.[10] Reinhold and Eilam,[11] working with sunflower hypocotyl, suggested that active transport operated in the absence of DNP but that in its presence sugars diffused into the cells.

The results presented herein suggest a coupling between glycolysis and active sugar uptake. Fermentation accompanied sugar uptake even when O_2 was not limiting. (Younis *et al.*[12] attributed an increased evolution of CO_2 in radish slices to a saturation of the respiratory enzymes. This is not the case with scutellum slices since the consumption of O_2 was reduced in the presence of 10 mM sugar.) Furthermore, the fact that uptake proceeded under N_2 indicates that glycolysis can support sugar uptake. This association between fermentation and uptake is the only evidence for a coupling between glycolysis and uptake. Fermentation also occurred when glucose was added to slices pretreated with uranyl nitrate (unpublished results). Since uranyl ion is presumed to inhibit active sugar uptake, this result indicates that fermentation (in air) is not obligatorily coupled to active sugar uptake. No evidence has been obtained to show that a specific glycolytic step is responsible; however, the data are consistent with the phosphotransferase system in bacteria where PEP is the energy source for uptake.[9] Garrard and Humphreys,[4] studying control of glycolysis in the maize scutellum, suggested that sucrose synthesis, utilizing ATP and producing ADP within the glycolytic compartment, triggered fermentation. The direct use of PEP in the sugar uptake process would by-pass, in part, the ADP requirement of glycolysis, and, thereby, might increase the ADP level of the glycolytic compartment resulting in a more rapid rate of glycolysis and fermentation. It may be that among various plant tissues the criterion which determines whether or not fermentation occurs is the relative rates of glycolysis and sugar uptake. A coupling of the two processes would be masked if sugar uptake proceeded at a slow rate relative to glycolysis.

The sucrose uptake vs. concentration curve of Fig. 1 closely fits a Michaelis–Menten curve, and this type of data is often presented in support of a carrier uptake mechanism. However, since sucrose uptake was nearly linear with time even though the external sucrose concentration was initially well below that required for saturation of the uptake mechanism and was declining rapidly during the course of the uptake, kinetics more complicated than the pseudo first order kinetics of the Michaelis–Menten type are indicated. Regardless of the mechanism of uptake, be it diffusion or a carrier-mediated active process, the rate would be expected to decrease as the sugar concentration in the solution was reduced as a result of uptake. When the sugar concentration was maintained at a constant level sucrose uptake increased with time whereas glucose uptake did not. Possible schemes to explain these phenomena follow. The rate of sucrose uptake is governed by at least two factors, the external concentration of sucrose and the internal concentration of a phosphate donor. When sucrose uptake begins, the phosphate donor is limiting; but, as uptake proceeds, fermentation generates additional phosphate donor which in turn increases the rate of uptake. Thus, the uptake of sucrose is autocatalytic. This is supported by data which show an increasing rate of CO_2 evolution with time in sucrose (Fig. 4). An increasing internal rate of glycolysis providing an increased internal concentration of the phosphate donor may have offset the effect of a decreasing outside sucrose concentration and maintained a higher than expected rate of uptake. It is not clear, however, how these factors combine to yield a constant rate of uptake.

[10] A. ROTHSTEIN and J. VAN STEVENINCK, *Ann. N.Y. Acad. Sci.* **137,** 606 (1966).
[11] L. REINHOLD and Y. EILAM, *J. Exptl Bot.* **15,** 297 (1964).
[12] A. E. YOUNIS, M. E. YOUNIS and M. A. GABR, *Plant Cell Physiol.* **10,** 575 (1969).

A similar scheme can be devised for glucose. With glucose uptake, the amount of diffusion of glucose into the cytoplasm could determine the rate of active transport by creating a competition in the cytoplasm for phosphate donors (ATP or PEP) between hexokinase (which phosphorylates glucose entering by diffusion) and the active uptake process. As the external concentration of glucose declines, the amount of glucose entering by diffusion declines, thereby allowing more phosphate donors to be available for the active uptake process. Thus, as the concentration declines, the active process has available a lower external concentration of glucose but a higher internal concentration of phosphate donor to drive the uptake process. The overall result is an uptake rate that is almost constant until the glucose in the bathing solution is depleted. At constant glucose concentration the amount of glucose entering by diffusion is constant and the overall rate of uptake remains constant.

A combination of active and passive glucose uptake mechanisms fits well with the role of the scutellum in germination. During the initial stages of germination when the glucose concentration available to the scutellum is low, the system is capable of quickly removing all of the available glucose; however, when the endosperm glucose is high, the scutellum can take up glucose in excess of the capacity of the active process.

A sugar uptake system coupled to glycolysis also fits the role of the scutellum. The ability to take up sugar under limited O_2 supply would be of obvious advantage to a seed under soil conditions. Garrard and Humphreys[4] have measured an RQ of 3 with whole scutella in air indicating that the scutellum itself imposes conditions of limited O_2 availability.

EXPERIMENTAL

Plant material. Maize grains (*Zea mays* L., cv. Funks G-76) were soaked in running tap H_2O for 24 hr and then placed on moist filter paper in the dark at 24–25° for 72 hr. The scutella were excised and cut transversely into slices 0·5 mm or less in thickness. The slices were washed in H_2O until the washings remained clear, blotted on filter paper and weighed in groups of 0·10–1·0 g depending on the type of experiment. Unless otherwise noted, incubations were carried out with 1·0 g of slices in 10 ml at 30° with rotation of solutions at *ca.* 200 rpm.

Analysis of sugars. Glucose and sucrose were determined by the glucose oxidase method (Glucostat Worthington Biochemical Corp., Freehold, N.J.). Samples for sucrose analysis were incubated for 2 hr with and without invertase prior to analysis. Fructose and reducing disaccharides were analyzed according to the Nelson–Somogyi copper reduction method[13,14] as reported by Spiro.[15] The alternate copper reagent suggested by Somogyi was used. When sampling the bathing solutions for sugars, amounts 0·1–2·0 ml were taken depending on the sugar concentration, and appropriate dilutions were made so that 0–140 μg of glucose or fructose were used for analysis. Twice this amount was used for disaccharides other than sucrose. The analysis of tissue sucrose has been described previously.[16] Uptake was measured by measuring the disappearance of sugar from the bathing solution.

Manometry. Experiments were carried out in a Warburg respirometer at 30°. The direct method for CO_2 was used.[17] The amount of tissue added to the flasks was 100 mg. When the slices were prepared, they were placed without weighing into 25 ml Erlenmeyer flasks in 10 ml of water and incubated for 1 hr at 30°. The water incubation removed leakable sucrose.[18] Following incubation, the slices were blotted and weighed into Warburg flasks. The sugar solutions were added from the side arm during the course of gas exchange measurements.

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