Sugar Transport in the Maize Scutellum

By

JOSEPH HENRY WHITESELL

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Joseph Henry Whitesell

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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 depleted even at concentrations well below those which saturated the uptake mechanisms. The effect of DNP, phloridzin, uranyl ion, and anoxia was to inhibit the uptake of sucrose approximately twice as much as the uptake of hexoses. Maltose was taken up without hydrolysis. Turanose was not taken up but slightly inhibited the uptake of sucrose. The following conclusions are drawn. (a) Sucrose is taken up actively without inversion. (b) Hexoses are taken up by two processes operating simultaneously, diffusion and active transport. (c) The active uptake mechanisms for sucrose and the hexoses are located at the plasmalemma. (d) The active uptake mechanisms for both sucrose and the hexoses are driven by glycolysis. (e) Metal binding characteristics of the scutellum are different from those of yeast in that binding is not specific to the uptake sites and bound metal ions are apparently not released during sugar uptake.

INTRODUCTION

Much research has been conducted on the movement of sugars in the cells of animals and microorganisms. Stein (1) reviews this work in a book which emphasizes the movement of nonelectrolytes across cell membranes. Much less work has been done on sugar transport in higher plants, and yet the problem is central to the understanding of plant physiology. At least with respect to higher organisms the movement of disaccharides is primarily a plant problem since the gut of animals takes up only monosaccharides (2). Sucrose is by far the most common carbohydrate translocated in plants (3).

Kursanov says, "A profound understanding of the laws of movement of substances is also of direct importance in agriculture, for it is precisely as a result of the transport of substances that growing tissues are provided with food and there is an accumulation of reserve materials in seeds, fruits, roots, and other parts of plants that are of prime importance in crop yields" (3, p. 213). Hartt et al. add a quantitative note, "Since sugarcane plants in Hawaii translocate sucrose at the rate of over one million tons per year, translocation studies have an immense economic potential" (4, p. 305). According to Hartt translocation may form a bottleneck in the total production of photosynthate.

Kursanov (3) points out that the carrier theory which has been developed with respect to mineral elements by Epstein and others is probably applicable in the case of the transport of sugars but factual

material to support such concepts is still greatly inadequate. He also states that the very existence of carriers has not been proven. The studies of Bieleski (5) on the accumulation of sugars by phloem tissue add support to the contention that an active movement of sucrose is probably a part of phloem transport.

The work reported herein was undertaken to gain a better understanding of the process of sugar uptake, especially that of sucrose. Several characteristics make the corn scutellum well suited for the study of sugar uptake: (a) The uptake of sugars is rapid allowing relatively short time periods to be used; (b) carbohydrate is stored in the form of sucrose rather than starch; glucose is not accumulated; (c) there is no ageing phenomenon during which an adjustment in the respiratory rate occurs; and (e) morphologically, the scutellum is leaf tissue which functions in the movement of sugar from the endosperm to the developing seedling.

LITERATURE REVIEW

Several systems have been described in which sucrose is taken up without prior inversion.

Weatherley (6) measured sucrose uptake by floating leaf disks of Atropa belladonna on 10% sucrose solutions and taking dry weights before and after uptake. At times the amount of hydrolysis was very low and the amount of hydrolyzed sucrose in the bathing solution varied independently of uptake. Whereas pH affected the amount of hydrolysis it had very little effect on uptake. The amount of hydrolysis was greater when older leaves were used. Washing disks prior to treatment decreased the amount of hydrolysis. He concluded that sucrose was absorbed as such. He found (7) that the uptake of glucose and sucrose on a per mole basis was nearly identical.

Weatherley (8) also found that the uptake of sucrose was reversibly inhibited about 75% under nitrogen. He followed the loss and uptake of water as well as dry weight and included a discussion of the osmotic situation prevailing in the tissues. He concluded that the uptake of sucrose was probably an active process.

Experiments by Pennell and Weatherley (9) showed that sucrose uptake was inhibited about 50% by 2,4-dinitrophenol (DNP) and not at all by phloridzin (both at 1 mM). In this paper the amount of dry weight increase was shown to be caused partly by an increase in sucrose, glucose and fructose. The amount of increase not due to these sugars was assumed to be due to starch formation. Because a significant

amount of weight increase was due to starch it was argued that no "uphill" movement of sucrose need occur.

Porter and May (10) worked with tobacco leaf disks and measured uptake, accumulation and gas exchange in 5% solutions of sucrose and invert sugar. When invert sugar was supplied, glucose disappeared at a faster rate than fructose. Invert sugar caused a more rapid accumulation of starch than did sucrose. The total uptake of sugar was the same whether invert sugar or sucrose was supplied. Asymmetrically labeled sucrose could be recovered from the leaf disks after being applied in the bathing solution showing that sucrose could be taken up without inversion. They found an RQ of 0.74 during incubation of tissue in water, but this increased to about 1.1 when the tissue was incubated in sucrose or hexose. The specific activity of the CO_2 evolved was about the same as that of the applied sugar leading them to believe that the observed rate of gas exchange was not the result of a high rate of fermentation superimposed on the gas exchange in water. They suggested rather a shift in substrate to sugar when sugars were added to the bathing solution.

Vickery and Mercer (11) studied the uptake of sucrose by bean leaf tissue. The bathing solution contained only sucrose and a trace of fructose after incubation, indicating that extracellular hydrolysis was small or nonexistent. When tissue samples were analyzed most of the sugar was sucrose with small amounts of reducing sugars present. Gas exchange was measured and the RQ increased from about 0.8 to 1.0 when sucrose was added to the bathing solution. They noticed a linear phase of sucrose uptake with time despite large concentration differences in the bathing solution. The steady rate of sucrose uptake was

about 9 umoles/g fr wt hr from 1% (0.028M) sucrose. An inhibition of sucrose uptake of 55% was noted with 2.4×10^{-5}M DNP. Uptake was measured over periods of 8 to 26 hr.

Vickery and Mercer (12) reported increases in O_2 consumption upon the addition of sucrose that were of short duration and independent of concentration. They stated that the rate of CO_2 production showed no correlation with the concentration of sucrose in the external solution (and hence, in the free space) but was strongly correlated with concentration of sucrose in the apparent osmotic volume. This is used as an argument that the sites of carbohydrate metabolism are included in the osmotic volume for sucrose and that part, at least, of the cytoplasm is included within the membranes involved in sucrose transport. Sucrose accumulated against a gradient and the initial rates of sucrose uptake followed a concentration vs uptake curve adaptive to Michaelis and Menten kinetics. After several hr of uptake the rate decreased. This decrease was attributed to inhibition of further uptake by sucrose inside the cell. They argued against the possibility that sucrose pumps occur only at the tonoplast.

Hardy and Norton (13) studied the uptake and utilization of ^{14}C-labeled sucrose, glucose and fructose by slices of potato tubers. Glucose was taken up faster than fructose. All three sugars were found when untreated tissue was analyzed for sugars. It was suggested on the basis of the labeling of various intermediates that sucrose was absorbed unchanged and transported to storage where part of it was hydrolyzed resulting in storage of all three sugars.

Sacher (14), using bean pod tissue, found an extracellular or outer space invertase which varied seasonally in its activity. However,

sucrose uptake was not dependent on invertase activity as shown by the observation that sucrose was taken up in the absence of outer space invertase activity. Glucose uptake was three times as fast as fructose uptake from 0.03M solution. There was no effect of 10^{-5} to 10^{-3}M uranyl nitrate on uptake of 0.03M glucose or sucrose. The fact that sucrose was taken up as such was also demonstrated by showing that the glucose/fructose radioactivity ratio was little changed by uptake when using fructose-labeled sucrose. Uniformly labeled sucrose remained uniformly labeled even in the presence of unlabeled fructose or glucose in the bathing solution. DNP (5×10^{-4}M) inhibited uptake from 0.0003M sucrose 96% and from 0.03M sucrose 88%. In freshly cut tissue the endogenous sugars consisted largely of glucose and fructose, and only trace amounts of sucrose. Upon incubation in sugar solution sucrose was stored, apparently in the vacuole, and the sucrose/reducing sugar ratio was high. However, after incubating the tissue for 16 hr in water the sucrose was hydrolyzed and the ratio greatly decreased. Sacher argues on the basis of several lines of evidence that the rate limiting step in hexose uptake lies in the formation of sucrose and that the cytoplasm is free space to the hexoses.

Kriedemann and Beevers (15, 16) worked with castor bean seedlings. In the germinating castor bean, sucrose is synthesized in the endosperm at the expense of fat. The sucrose is taken up by the cotyledons, and it is transported from them into the developing seedling axis. In the castor bean cotyledons, which are functionally analogous to the scutellum, most of the sugar is in the form of sucrose with virtually no free hexoses present. They showed a linear uptake of sucrose with time by the cotyledons and sucrose was accumulated against a concentration

gradient. DNP partially inhibited the uptake of sucrose. Sucrose was shown to be taken up without inversion by several lines of evidence including the retention of asymmetry of labeled sucrose applied in the bathing solution. In this tissue sucrose was taken up at a higher rate than either hexose and, more unusual, the rate of fructose uptake was considerably greater than that of glucose uptake.

Kriedemann (17), on the basis of microautoradiographs of castor bean cotyledons exposed to labeled sucrose for 20 min, suggests that the cell walls and intercellular spaces provide a diffusion pathway by which solutes can gain access to the vascular system from an external source.

Kursanov (3) refers to work in which it was shown that the fibrovascular bundles from sugar beet petioles took up sucrose, glucose and fructose but exhibited a much higher affinity for sucrose.

Grant (18) studied the uptake of glucose, fructose, and several other monosaccharides by carrot and corn root tissue. Some of his results were as follows. Carrot root tissue exhibited a lag of several hr before uptake began. The uptake of glucose under N_2 was less than 25% of that in air. He showed that glucose entered the cell and accumulated as the free sugar against a concentration gradient. The glucose concentration in the carrot tissue exceeded 0.05M assuming equal distribution within the fresh weight of the tissue. Uptake of glucose proceeded at a constant rate from 0.001M solution until the bathing solution was exhausted. He showed that the uptake of glucose and several other sugars follows uptake vs concentration curves according to Michaelis and Menten kinetics. He did not seem to be concerned with the fact that a constant rate of uptake with time, in spite of a

declining sugar concentration, is inconsistent with reaction rates as predicted by Michaelis and Menten kinetics. The maximum rates of sugar uptake by carrot discs were from 3 to 10 umoles/g hr and from corn roots were from 7 to 34 umoles/g hr (19). It was shown that in carrot tissue the respired CO_2 was derived preferentially from the entering sugars.

ApRees and Beevers (20) using carrot and potato slices measured O_2 consumption and CO_2 evolution in low (0.5 umoles/ml) concentrations of glucose. The RQ did not vary significantly from unity and the addition of glucose did not induce significant changes in O_2 uptake or CO_2 output.

Reinhold and Eshhar (21) demonstrated an active uptake mechanism in carrot root tissue which was capable of accumulating 3-o-methylglucose. The rate of uptake of 3-o-methylglucose varied with concentration approximately as predicted by Michaelis and Menten kinetics. After a period of uptake the tissue was rinsed for 30 min and then placed in water and a concentration ratio of 75:1 was maintained between the concentrations in the tissue and water. Chromotography and analysis of CO_2 indicated that 3-o-methylglucose was not metabolized.

Harley and Jennings (22) studied the uptake of sugars by beech mycorrhizas. They found that the rate of uptake vs concentration curves for glucose and fructose formed rectangular hyperbolas but the shapes of the curves were different, the maximum rate for fructose being higher than the maximum rate for glucose. In this tissue there is considerable hydrolysis when sucrose is supplied. DNP markedly inhibited the absorption of hexoses. When mixtures of glucose and fructose were supplied, glucose was preferentially absorbed. The addition of sugars caused a respiratory stimulation and from equimolar

concentrations the stimulation caused by glucose was considerably greater than that caused by sucrose in spite of the fact that a roughly equal amount of sugar on a weight basis was taken up during the measurements.

Morgan and Street (23), studying the carbohydrate nutrition of excised tomato roots, found an RQ of about 0.75 in water and about 1.0 in sucrose. The root segments had been starved prior to measurement and the uptake of O_2 was stimulated by the addition of sugars. The RQ of root tips supplied with sucrose, dextrose, galactose, or raffinose was within the range 0.90-0.96. The endogenous respiration had an RQ as low as 0.70 and in mannose as low as 0.60.

Thomas and Weir (24) measured the uptake of sugar by tomato root segments from solutions of 0.05M glucose and 0.025M sucrose. It was found that more sugar on a weight basis was taken up when sucrose was supplied as compared to glucose. Sucrose is markedly superior to glucose in supporting growth of excised tomato roots.

When radish root slices are incubated in sucrose there is a considerable amount of extracellular hydrolysis (25). It is not known whether or not any sucrose is taken up without inversion. Sucrose at 0.029M and 0.058M both stimulated the evolution of CO_2 and it was suggested that this was due to a saturation of respiratory enzymes.

Bieleski (5) measured the uptake of sucrose by excised vascular bundles or phloem tissues from a variety of plants. In apple phloem and celery vascular bundles about 70% of the uptake from ^{14}C sucrose could be found in the tissue in the form of sucrose. Sucrose uptake, from 0.001 and 0.01M solution, proceeded at a progressively slower rate until the external solution contained about 10% of the original amount of sucrose.

Sucrose was taken up against concentration gradients of the order of 10^3. The rates of accumulation by vascular bundles or phloem tissue were much higher than rates by parenchyma from the same plant. Vascular tissue accumulated sucrose at rates of 9 to 16 umoles/g fr wt hr from 0.1M sucrose solution.

Reinhold and Eilam (26) addressed the question as to whether there was a diffusion barrier between external substrate and sites of respiration. They supplied sunflower hypocotyl segments with labeled glucose or glutamic acid in the presence or absence of DNP and measured the total amount of CO_2 evolved and its specific activity. They subjected the data to kinetic analysis and concluded that there was not an effective diffusion barrier between the external substrate and the sites at which substrates are respired. An alternative is given; "It remains just possible, however, that such a mechanism [active transport] does operate in the absence of DNP, but that in its presence the molecules are able to diffuse freely inwards owing to a disorganization of the cell membranes" (p. 306).

Sugar cane is probably the most studied of higher plants in regards to sugar movements. Bieleski (27, p. 204) stated the uptake problem as follows: "...it was found that disks of sugar cane tissue placed in aerated distilled water lost very little of their endogenous sugar to the water. Thus either the tonoplast is extremely impermeable to sugar movement or there is an accumulation mechanism in the cell which actively opposes the outward diffusional movement of sugar. The first is perhaps the simpler explanation, but raises the problem of explaining how the sugar originally became accumulated behind the impermeable tonoplast."

Sugar cane exhibits a large, rapid (1-hr duration, 8-min half time), apparent-free-space uptake followed by a slow uptake which can occur against a gradient and which results in sugar accumulation. The accumulation uptake will proceed over a period of 72 hr. In comparing rates of uptake of various sugars he found that glucose uptake was more than double that of sucrose uptake on a molar basis. Uptake of fructose was similar to that of glucose.

Bieleski measured respiration during sugar uptake and found an increased O_2 uptake upon the addition of sugar to the bathing solution. He does not mention any change in RQ associated with sugar uptake.

Bieleski (28) found that sugar accumulation was completely inhibited by 10^{-5}M DNP. Phloridzin at 2×10^{-3}M caused from 10 to 80% inhibition of the uptake of glucose. When tissue was prewashed in 2×10^{-3}M magnesium chloride it caused a 0-20% increase in the amount of glucose accumulated. Double reciprocal plots of sucrose, glucose and fructose uptake rates vs concentration yielded straight lines (29). The V_{max} reported for sucrose was 0.7 umoles/g hr.

Glasziou (30, 31) suggested that the outer space consists of the cell walls and cytoplasm and is in diffusion equilibrium with the external solution. "Hence the cytoplasm is part of the outer space where outer space is defined as the tissue volume which comes to rapid diffusion equilibrium with sugars in the external solution (the outer and inner space for this tissue may be quite different for solutes other than sugars)" (31, p. 178). Tracer studies showed that the hexoses in the inner space came from hydrolysis of stored sucrose.

Hatch et al. (32) reported on some of the enzymes involved. They report characteristics of sucrose synthetase in the direction of su-

crose synthesis. Activity in the reverse direction could not be detected because of the presence of a phosphatase which rapidly hydrolyzed UDP to UMP. Evidence for the presence of sucrose-P synthetase was presented, and acid and alkaline invertases were described. They could not find sucrose phosphorylase. Enzymes for the synthesis, interconversion, and breakdown of hexose phosphates were identified. The amounts of acid and alkaline invertases vary with the growth rate and the sucrose storage rate (33) suggesting a key role for invertase in regulating the movement and utilization of sucrose. Sacher et al. (34) present a scheme for the sugar accumulation cycle in immature sugar cane. Acid invertase occurs both in the outer space and the storage compartment. Sucrose is hydrolyzed prior to uptake and glucose is taken up several times as fast as fructose. Sucrose is released from storage via hydrolysis and diffusion of the hexoses out of storage.

Hatch (35) demonstrated the presence of sucrose-P synthetase in both leaf and storage tissue of sugar cane. He also showed the synthesis of sucrose-P by tissue supplied with glucose. Sucrose was stored more rapidly from sucrose than from sucrose-P and more rapidly from fructose than from fructose-P. This is consistent with the proposition that sugar phosphates do not penetrate membranes as easily as do non-phosphorylated sugars. Asymmetry of labeled sucrose was lost during storage. While only small quantities of sucrose were stored when sucrose-P was supplied, the asymmetry of label was largely maintained. This was consistent with a scheme in which sucrose-P is formed by the action of sucrose-P synthetase and sucrose is stored against a sucrose concentration gradient via the hydrolysis of sucrose-P to yield stored sucrose.

In further support of such a scheme Hawker and Hatch (36) demonstrated the presence of a specific sucrose phosphatase in sugar cane, carrot roots, etiolated barley, oat, and pea seedlings, parsnip root and potato tuber. The enzyme was associated with particles which behaved like mitochondria during differential centrifugation. Mendicino (37) had earlier described enzymes in wheat germ and green leaves that included sucrose synthetase, sucrose-P synthetase, and a nonspecific sucrose phosphatase.

Hawker and Hatch (38) present a scheme for the mechanism of sugar storage in mature sugar cane tissue. Evidence was presented to show that the hydrolysis of sucrose is a prerequisite to storage and a rate limiting step. Mature cane tissue contains an acid, wall-bound invertase and a neutral invertase apparently located in the cytoplasm. The storage compartment invertase found in immature tissue is absent in mature tissue. Sucrose storage takes place more rapidly from hexoses than from sucrose. Uptake of both glucose and sucrose as a function of the concentration of the bathing solution had the kinetic properties of an enzyme-catalyzed reaction. In studies on the localization of enzymes it was found that most if not all of the sucrose synthetase was located in the conducting tissue, and it is pointed out that it may function in the breakdown of sucrose.

Hatch and Glasziou (39) presented direct evidence that sucrose is the predominant component of translocated photosynthate in sugar cane. The asymmetry of labeled sucrose was maintained through the vascular tissue of the leaf, sheath, and stem. Randomization did occur during movement into storage.

Sacher (40) presented an argument for extracytoplasmic sucrose

synthesis in the bean endocarp. He supplied UDPG and labeled fructose and obtained sucrose in which the label was predominately in the fructose moiety. Experiments also indicated the presence of UDPG pyrophosphorylase in the extracytoplasmic space. When labeled fructose was supplied and sucrose synthesis occurred in the cytoplasm the ^{14}C glucose/^{14}C fructose ratio was approximately one; this sucrose remained in the tissue even after extensive washing.

By using acetone-extracted chloroplasts from sugar cane, Haq and Hassid (41) were able to show the synthesis of sucrose-P from UDPG and fructose-P and the synthesis of sucrose from UDPG and fructose. The preparation contained phosphatases that hydrolyzed sucrose-P and fructose-P.

Schoolar and Edelman (42) measured secreted sugar, CO_2 fixation, sugar formation, and starch synthesis by leaf disks of sugar cane floated on various solutions. The amount of sucrose secreted into the bathing solution was increased by 10^{-4}M sodium iodoacetate (IOA). About one-third of the total sucrose synthesized during a 4-day period was secreted. The inhibitor caused no change in the amount of soluble sugar within the disks and it caused an increase in the amount of total soluble photosynthate produced. Respiration measured in the dark showed an RQ of considerably less than 1.0 and this was reduced even further by IOA. Other respiratory inhibitors did not elicit similar responses.

Many investigations have been made of various enzymes involved in sugar transformations. Only a few will be mentioned here. In his review article on sugar transformations in plants, Hassid (43) discussed the characteristics of sucrose synthetase and sucrose-P syn-

thetase, the two enzymes most likely to be involved in the synthesis of sucrose from glucose and fructose or from either hexose alone.

Putman and Hassid (44) studied the transformation of sugars in vacuum-infiltrated disks of Canna leaves. When labeled fructose or glucose was provided, labeled sucrose was recovered which was labeled in both hexoses; however, no free labeled glucose could be found when labeled fructose was provided and vice versa, an indication that sucrose was formed via phosphorylated hexose intermediates. When sucrose was provided in the bathing solution there was rapid inversion of the sucrose with the appearance of hexoses in the bathing solution followed by a resynthesis of sucrose within the tissue.

Cardini et al. (45) point out that the equilibrium constant of sucrose phosphorylase lies in the direction of sucrose hydrolysis and that sucrose phosphorylase has not been found in higher plants. A study of the characteristics of sucrose synthetase from a variety of plant tissues is reported. The equilibrium constant, K = (sucrose x UDP)/(UDPG x fructose), varied from 2 to 8 at 37° and pH 7.14 in different experiments.

Leloir and Cardini (46) studied the properties of sucrose-P synthetase but point out the difficulties caused by the presence of interfering enzymes (phosphatase and sucrase) when working with this enzyme.

Hawker (47) found that the ratio of sucrose-P synthetase/sucrose synthetase varied in different plants from 10 to 0.6.

Ricardo and ApRees (48) measured the activity of both acid and alkaline invertases and sucrose content during development of carrot and during ageing of root disks. There was a negative correlation

between sucrose content and acid invertase activity. Acid invertase activity was high during times of high sugar usage and low during times of high sucrose storage. They suggest that high invertase activity prevents sucrose storage and that during periods of low hexose demand hydrolysis is due to alkaline invertase which is not associated with the vacuole but located in the cytoplasm. They suggest that the acid invertase is located in the wall and at the tonoplast.

Kursanov et al. (49) compared the localization and properties of hexokinase with uptake characteristics of conducting tissues from sugar beet. This tissue takes up glucose faster than fructose and the hexokinase associated with the structural elements of the cells has a higher affinity for glucose than for fructose. On this basis they suggest that hexokinase on the membrane may be part of the uptake process.

The uptake of sugars and the metal binding characteristics of yeast have been studied intensively.

Rothstein (50) presents several lines of evidence to show that uranyl ion affects the uptake of glucose by yeast due to its binding to the surface and not to an uptake into the cytoplasm of the cell. Rothstein and Meier (51) describe the competition for uranyl ion between the yeast complexing loci and various complexing agents added to the bathing solution. On the basis of this work they concluded that the binding sites on the surface of the yeast cells were polyphosphates.

Uranium blocks about 90% of the uptake of glucose in yeast (50). Several other cations, including Co^{2+}, Mg^{2+}, Ca^{2+}, and Mn^{2+}, bind to the surface of yeast cells but uranyl ion forms a much more stable complex (52). Data were presented to show that whereas the other cations were bound to the same sites that bind uranyl ion, they did not inhibit the uptake of glucose.

Data showing the amount of various ions bound to the surface of yeast cells as a function of the ion concentration are also presented by VanSteveninck and Booij (53). They showed that in the case of Ni^{2+} or Co^{2+} when glucose was added to the cells the metal was displaced from the surface of the cells and appeared free in solution. When the glucose had been taken up by the cells the metals were again bound. If cells were first poisoned with IOA and then supplied with glucose a small amount of glucose uptake occurred but was complete in 15 min. The amount of glucose uptake by the poisoned cells was the same (on a umole basis) as the amount of uranyl ion bound (on a uequivalent basis) by nonpoisoned cells. It was possible by adjusting the growth medium to vary the amount of phosphorus per yeast cell without causing irreversible damage to the cells. The amount of uranyl ion bound and the amount of glucose taken up after poisoning were both reduced in phosphorus deficient yeast. There was a good correlation between the amount of uranyl ion bound and the amount of glucose taken up by poisoned cells. When yeast was poisoned and then provided with glucose the uranyl binding capacity disappeared. The addition of IOA alone caused a 50% inhibition of cation binding which could be reversed by washing the cells in water.

VanSteveninck and Rothstein (54) present an argument to show that in yeast, sugar uptake can proceed by facilitated diffusion or by an active uptake mechanism. The facilitated diffusion system can be demonstrated with galactose uptake by uninduced cells and with glucose uptake by poisoned cells. The active transport system is driven by the energy released by glycolysis in spite of the fact that glucose is quickly utilized and does not accumulate in the cells. The two

systems are different with respect to Ni^{2+} binding, effect of Ni^{2+} on uptake, concentrations of uranyl ion required to inhibit uptake, kinetic parameters, and patterns of specificity.

Rothstein and VanSteveninck (55) summarized work done on uptake and metal binding by yeast cells. It was pointed out that the inhibitory effects of uranyl ion and Ni^{2+} are not due to displacement of a required cation. The conclusion is reached that the phosphoryl sites, to which uranyl ion binds, are used continuously in glucose transport and are regenerated continuously by glycolysis. In the yeast system a close correlation is pictured between glycolysis and uptake and glycolytic ATP is assumed to be the energy source for driving uptake. Carrier and glycolytic reactions are thought to be in close geographic proximity. In their scheme to explain the transport of sugars the carriers for facilitated diffusion and for active transport are considered to be the same. When active transport occurs the amount of carrier available for facilitated diffusion is reduced.

Wheeler and Hanchey (56) placed oat roots into 0.1 and 1.0 mM uranyl acetate for varying periods of time and then made electron micrographs in which crystals, apparently composed of a uranium complex, could easily be seen. After a 30-min treatment followed by a 30-min desorption the uranyl complex was sharply localized in cell walls, intercellular spaces and secretory products in direct contact with cell walls. With longer treatment times, up to 20 hr and the lower concentration, the uranyl complex crystals could be found in vesicles in the cytoplasm and in the vacuole. Otherwise the cells were normal with no uranyl ion free in the cytoplasm or in cell organelles. Uranyl ion apparently caused a definite dilation of the membranes from a normal

width of 90 Å to a width of from 150 to 200 Å. This effect could be seen on the plasmalemma of treated cells and in vesicles which contained uranium. They concluded that few, if any, free uranyl ions passed through the protoplast and that uranyl ion in addition to being bound to the plasmalemma is bound to cell walls and to secretory products along its surface.

Roseman (57) has reviewed the literature on a bacterial phosphotransferase system that is thought to be responsible for the uptake of sugars. The system as it operates in Escherichia coli consists of three protein fractions: Enzyme I, Enzyme II, and a low molecular weight protein designated HPr. Phosphoenolpyruvate (PEP) is the phosphate donor, and a variety of sugars including some disaccharides can serve as acceptors.

Enzyme I and HPr are found in the cytoplasm and Enzyme II is associated with the membrane. Enzyme I and HPr are constitutive whereas Enzyme II is constitutive with respect to glucose. Most Enzymes II are inducible. The specific sugar requirements of the system are due to Enzyme II. Enzyme I and HPr are common to all sugars phosphorylated by the system. Enzyme I catalyzes the transfer of phosphate from PEP to HPr which serves as a phosphate carrier. The specific Enzyme II then catalyzes the transfer of phosphate from phosphate-HPr to the sugar being phosphorylated. Roseman presents convincing evidence to show that the phosphotransferase system is the same as the permease systems and is responsible for the uptake of sugars. In most cases exogenous sugars enter the cell as sugar phosphates and this is described as group translocation. When free sugar enters the cell the process is called active transport.

Staphylococcus aureus accumulated sucrose-P when incubated in sucrose and it is thought that the phosphotransferase system is operative in the uptake and phosphorylation (58).

Edelman et al. (59) worked with scutella, roots and shoots of oats, rye, wheat, and barley. They showed that the scutellum contained a higher ratio of sucrose to hexose than did the root or shoot. Hexose absorption was inhibited by about half when experiments were run under nitrogen. Substantial sucrose formation took place in the scutellum under nitrogen, whereas incorporation into amino-acids, amides, malic acid, and sugar phosphates was considerably reduced. In these tissues fructose is absorbed at about half the rate of glucose. Sugar phosphates, sucrose, glucose, fructose, glutamic and aspartic acids and their amides, malic acid, CO_2, and polysaccharides were found to contain label after applying tracer amounts of labeled fructose or glucose. The scutellum was shown to contain much lower levels of hydrolytic enzymes than the roots or shoots. All of the enzymes necessary for the formation of sucrose from hexose were found in the scutellum and a scheme is presented to show the path of sucrose synthesis which involves the enzyme sucrose-P synthetase.

Humphreys and Garrard have published a series of papers dealing with the uptake, production, storage, and leakage of sugars by the corn scutellum. They demonstrated that glucose uptake proceeded at a constant rate even though glucose in the bathing solution was largely depleted of glucose as a result of uptake (60). The rate of glucose uptake was shown to vary depending on the conditions and length of the prior incubation of the tissue. Changes in the tissue content of various sugars and sugar phosphates after varying periods of time in

water were presented, and it was shown that mannose inhibited the uptake of glucose. Data were presented to show that the corn scutellum accumulates carbohydrate mostly in the form of sucrose, the content of starch and hexose being low.

Experiments concerning the glucose-free space of the scutellum, which involved measuring the amount of glucose in the tissue after incubation in various concentrations of glucose in the presence and absence of DNP and mannose and the measurement of glucose exit following transfer into water, indicated that the space was intracellular and that a carrier was not involved. Fructose and mannose occupied a space of similar size (61).

When incubated in high concentrations of fructose (0.1-0.9M), scutellum slices synthesized sucrose, some of which was stored and some of which leaked into the bathing solution (62). The leakage of sucrose was reduced in the presence of Mg^{2+}, Mn^{2+} or Ca^{2+} and EDTA increased the leakage from the synthesis compartment (63).

When sucrose storage was measured after incubation in fructose or sucrose it was found that the pH of the incubation medium was more important when sucrose was the sugar taken up (64). The maximum storage occurred at a pH of 4.5 with both sugars; at a pH of 7.5 there was about a 20% decrease in stored sucrose when fructose was the exogenous sugar whereas with sucrose in the bathing solution there was 80% inhibition. The authors suggested that the sites of sucrose uptake are in contact with the bathing medium. The amount of sucrose storage was increased when sucrose was added to the bathing solution containing optimum amounts of hexose. This would not have been the case if sucrose were being hydrolyzed prior to uptake or during the process of uptake.

The loss from storage was measured by loading the storage compartment with ^{14}C sucrose and then incubating the tissues at different pH values and with and without "cold" sucrose. More sucrose was lost at the higher pH values and the loss was greater in the presence of "cold" sucrose than in water indicating an exchange between external and stored sucrose.

The addition of fructose or glucose to scutellum slices (65) resulted in a strong, aerobic fermentation and the concommitant production of ethanol. Increased sucrose synthesis upon incubation in fructose accompanied an increase in glycolysis without an increase in O_2 uptake. This supported the idea that glycolytic ATP might be responsible for sucrose synthesis. When incubated in water the RQ for intact scutella was about 3 while that for slices was near unity.

It was concluded, on the basis of the levels of various phosphofructokinase regulators during different rates of glycolysis, that control of glycolysis in the scutellum was exerted through the availability of substrate and the distribution of adenine nucleotides and inorganic phosphate.

Pretreatment with tris(hydroxymethyl)aminomethane (tris) prevented the storage of exogenous sucrose but the inhibition could be reversed by hydrogen ion or by Al^{3+}, Mn^{2+}, and to a lesser extent Mg^{2+} and Co^{2+}. Sucrose storage from fructose was little affected by the pretreatment with tris (66).

Pretreatment with uranyl nitrate (67) was similar in its effects to pretreatment with tris in that storage of exogenous sucrose was inhibited and the inhibition could be reversed by H^+, Al^{3+} and to some extent Mn^{2+}. Uranyl ion pretreatment only slightly inhibited sucrose

synthesis from hexose and the inhibition was not thought to act through the uptake of hexose.

It is possible, by incubating slices in high concentrations of fructose, to build up considerable concentrations of sucrose in the synthesis compartment. That this is sucrose and not sucrose-P has been demonstrated. Upon reducing the external sugar concentration and inhibiting leakage, the free sucrose in the synthesis compartment will be transferred to the storage compartment. Experiments with mannose (68) indicated that whereas mannose inhibited the storage of exogenous sucrose, it did not affect the storage of sucrose which had accumulated in the synthesis compartment. The suggestion was offered that the storage of synthesis compartment sucrose involved a non-nucleotide phosphate doner such as occurs in the bacterial phosphotransferase system.

Pretreatment of scutellum slices with HCl (0.01M) did not inhibit the storage of exogenous sucrose or the synthesis and storage of sucrose from fructose (69).

Recently Humphreys and Garrard (70) suggested that leakage is from the sieve tubes and is the end result of a series of events which include intercellular sucrose transport, vein leading and phloem transport. Several compounds, all of which can either displace or form complexes with Ca^{2+} and Mg^{2+}, protect the leakage process which is labile at 30^{o} in water.

Since some confusion exists in the use of terminology concerning uptake studies, definitions of several terms used in the presentation of data and the discussion are given here.

Uptake--This term is used for the disappearance of a substance

from the bathing solution and is not meant to imply a particular mechanism. Some authors would use absorption.

Diffusion--The net movement of molecules as a result of their thermal motion from a region of higher to one of lower concentration. Where a membrane is crossed resistance may be due to the limited number and size of pores in the membrane or to the solubility characteristics of the solute in the membrane.

Facilitated diffusion--This is a process in which a concentration gradient is the driving force as in diffusion and the process leads to a disappearance of the gradient. The process is thought to involve a membrane constituent (carrier) located on or in the membrane which "facilitates" diffusion. Facilitated diffusion of a solute across a membrane requires no input of energy other than that needed to maintain structure, it may show a high degree of specificity, and kinetics are likely to show saturation thus not conforming to Fick's law of diffusion.

Active transport--This process involves the use of metabolic energy as a driving force. It is capable of bringing about the accumulation of a substance against its concentration gradient. It is generally characterized by a high degree of specificity and saturation kinetics.

METHODS AND MATERIALS

Preparation of Scutellum Slices

Corn grains (*Zea mays* L., 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 and cut transversely with a razor blade 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 into groups of from 0.1 to 1.0 g depending on the type of experiment.

During preparation, the slices were thoroughly mixed so that each group of slices was a random selection from 50-100 scutella. This resulted in excellent agreement when measurements of uptake or accumulation were made on duplicate groups of slices from one day's preparation. Results were not as consistent when duplicates were compared from different days' preparations.

Unless otherwise noted, incubations were carried out with 1.0 g of slices in each 25 ml flask at 30° in a "Gyrotory" water bath (New Brunswick Scientific Company, New Brunswick, N. J.) rotating at approximately 200 rev/min. The volume of solution was usually 10 ml.

Analysis of Sugars

Glucose and sucrose were determined by the glucose oxidase method (Glucostat, Worthington Biochemical Corp., Freehold, N. J.). Sucrose samples 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 (71, 72) as reported by Spiro (73). The alternate copper reagent suggested by Somogyi was used.

When sampling the bathing solutions for sugars, amounts between 0.1 and 2.0 ml were taken depending on the concentration, and appropriate dilutions were made so that between 0 and 140 ug of glucose or fructose were used for analysis. Twice this amount was used for disaccharides other than sucrose. Absorbance was read on a Klett-Summerson Model 8005 photoelectric colorimeter.

Tissue sucrose was extracted by pouring 20 ml of boiling 80% ethanol over the slices and continuing the boiling for 30 sec. The slices were steeped in the alcohol for 30 min, the alcohol was decanted and the procedure repeated. The slices were then rinsed three times with 5 ml portions of alcohol. The combined extracting solutions were evaporated almost to dryness on a steam bath. Water was added to a volume of 50 ml and 10 drops of 0.1N NaOH added to adjust the pH. The resulting aqueous solution was frozen. After thawing the solution was centrifuged for 10 min in a clinical centrifuge. For the determination of sucrose, 0.1 ml of this solution was used.

Manometry

Experiments were carried out in a Warburg Respirometer at 30°. The direct method for CO_2 was used (74). The amount of tissue added to the flasks was either 100 or 300 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 (69). Following the incubation the slices were blotted

and weighed into Warburg flasks. The sugar solutions were either added at the time the slices were placed in the flasks or added from the side arm during the course of gas exchange measurements.

Metal Analysis

Uranyl ion was determined by the method described by Rulfs et al. (75). Absorbance was read at 400 nm as suggested by Silverman et al. (76). Aluminum was determined by the method of Gentry and Sherrington (77) as reported under procedure A by Sandell (78). The extraction was made at pH 5. The purpurate method of Williams and Moser (79) as described by Sandell under procedure A (78) was used for the determination of calcium. Magnesium was determined by the Eriochrome Black T Method (80). The permanganate method of Nydahl (81) reported by Sandell (78) was used for the determination of manganese. Cobalt was determined by a modification of the Nitroso-R salt method of Marston and Dewey (82) as reported under procedure B by Sandell (78).

The methods used for uranyl ion and aluminum are not specific; however, since samples from untreated controls showed zero values interfering ions were not present. The methods used for manganese and cobalt are specific. Magnesium, in amounts that would be present in the solutions analyzed in these experiments, is reported not to interfere with the purpurate method for calcium. Copper, iron, and manganese do interfere to some extent. The value obtained for calcium in the control samples was low but not zero; however, some calcium would be expected to leak from the control slices. The method for magnesium is not specific, but the amounts of interfering metals in these experiments were too low to cause significant error. The amounts of calcium and magnesium found by using these methods agree very closely

with those found by atomic absorption spectroscopy in earlier work by Humphreys and Garrard (70).

RESULTS

In the first section kinetic data will be presented to show the rates of uptake of sucrose, glucose, and fructose with time and the variation of uptake rate with concentration of the bathing solution, it being assumed that sucrose uptake occurs without inversion. In the second section the assumption that sucrose is taken up as such will be justified and the effects of several inhibitors on sugar uptake will be presented. In the third section gas exchange data will be presented and it will be shown that fermentation accompanies sugar uptake. The fourth section presents the results of a study of metal binding characteristics of the scutellum slices and the effects of several cations on the uptake of sugars.

Kinetics

Figure 1 shows the cumulative uptake of sucrose from two concentrations of sucrose over a 2 hr period. In these experiments the first sample was taken 1 min after adding the sugar solution to the slices.

When the bathing solutions were analyzed for sucrose a small amount of glucose was usually found except in the samples taken at 1 min after adding the solution. This glucose could have come from the extracellular inversion of sucrose or from glucose diffusing out of the tissue following intracellular inversion of sucrose. Table 1 shows the data from which the upper two curves in Figure 1 were calculated. The solid line in Figure 1 represents data calculated (from column 6,

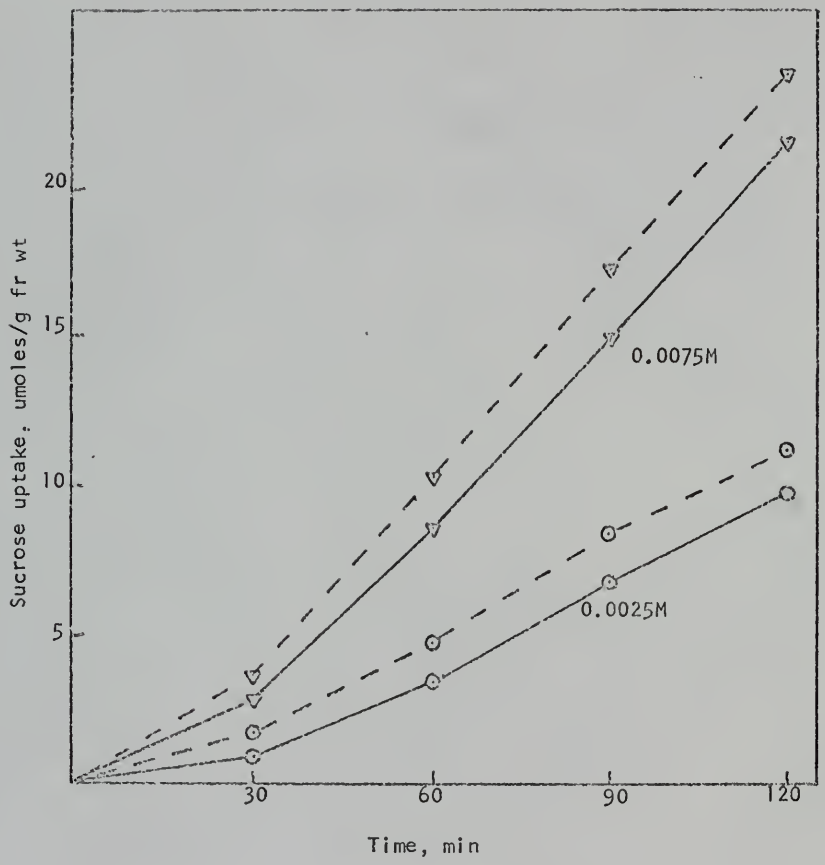


Figure 1. Sucrose Uptake Vs Time. One g of slices was used in each flask. The slices were incubated for 1 hr in water, given 1 rinse, and then 10 ml of the sugar solution was added. The first sample was taken 1 min after adding the solution (time zero on the graph). Samples of 0.5 ml were taken so that the volume during the last 30 min had been reduced to 8.0 ml.

Table 1

Method of Calculating Data*
(0.00750M sucrose)

	Klett units					
1	2	3	4	5	6	7
Time min	Without invertase	With invertase	Due to sucrose (3-2)	Decrease in sucrose due to uptake	Decrease in column 3	Concentration of sucrose M
0	0	88	88			0.00660
30	1	84	83	5	4	0.00623
60	3	76	73	10	8	0.00547
90	4	66	62	11	10	0.00463
120	4	55	51	11	11	0.00383

*Values shown in Figure 1 were derived by multiplying the figures in column 5 (dotted line) and column 6 (solid line) by the appropriate conversion factor.

Table 1) by ignoring the non-invertase-treated sample. In this case it is assumed that glucose is coming from extracellular inversion and the sucrose inverted but not taken up is not counted as uptake. The dotted line represents the actual decrease in sucrose of the solution (calculated from column 5, Table 1). The two methods do not result in large differences in calculated uptake. Table 1 is presented to demonstrate the method of measuring sucrose uptake. The remainder of sucrose uptake data presented was calculated by the method represented by the solid lines.

When the rinse water was withdrawn by suction immediately before adding the sucrose, an unmeasured amount of water adhered to the slices and to the sides of the flask and caused a dilution of the added sugar solution. There is probably also a free space volume in the slices which causes dilution so that analysis of the first samples indicated a sucrose concentration considerably lower than that which was initially added. This decrease in concentration can be accounted for by dilution. In the experiment of Figure 1, the concentration 1 min after adding the sucrose was 0.00660M when 0.00750M sucrose was added and 0.00225M when 0.00250M sucrose was added. These concentrations can be accounted for if it is assumed that there was 1.1 ml of water and free space in the case of 0.00250M sucrose. The figure for 0.00750M sucrose would be 1.4 ml. No effort was made to determine the amounts of water and free space. The problem was ignored by measuring uptake from the time the first sample was taken. The data are presented to show that there is not a large, rapid phase of uptake when sucrose is first added to the tissue.

The rate of uptake increases after the first 30 min. Either the

rate gradually increases over the first 30 min or there is a delay before uptake begins. In subsequent experiments the first sample was taken 15 min after adding the solution.

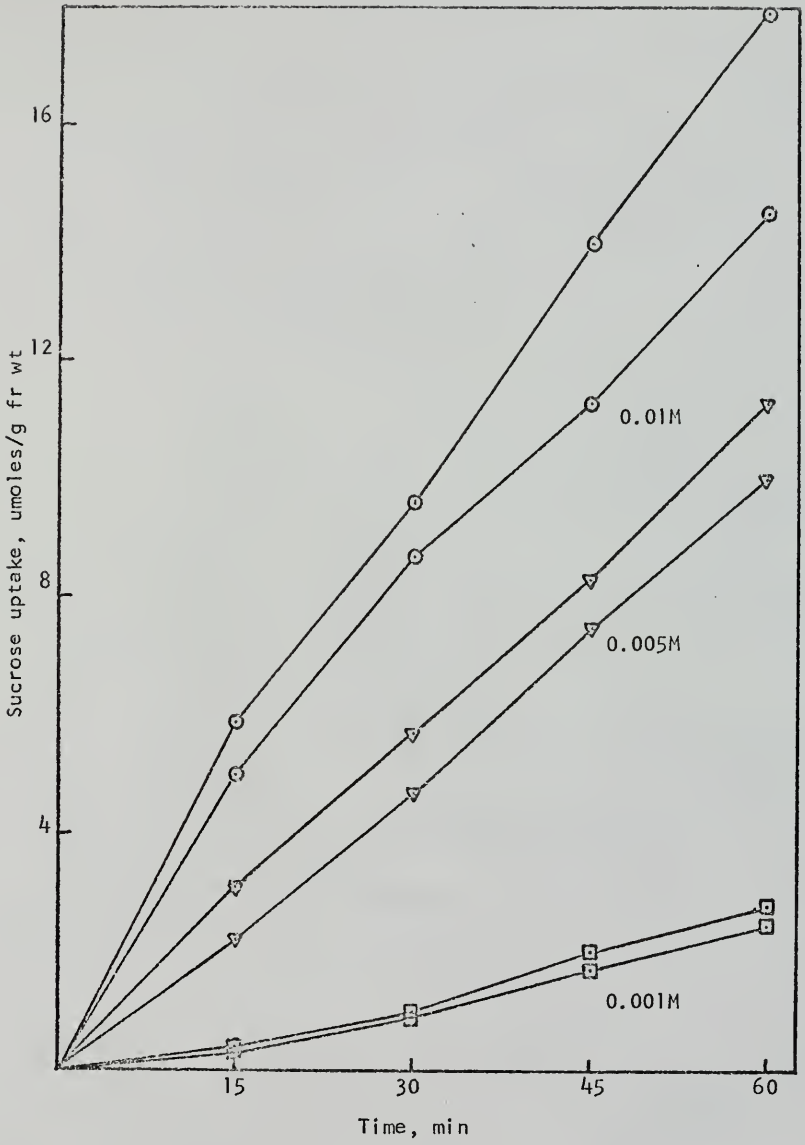
Notice that the rate of uptake was constant with time in spite of the fact that the concentration of the external solution was continually reduced. In the upper curve the concentration at the beginning of the last 30-min period was 70% of that at the beginning of the first 30-min period. In this same series of experiments a constant rate of uptake was obtained with 0.001M sucrose (data not presented) in spite of the fact that the concentration of the external solution was reduced to 40% of that added.

More experiments were run to determine the rate of sucrose uptake with time and the effect of sugar concentration on uptake rate. The rate of sucrose uptake with time is shown in Figures 2 and 3. The curves are more uniform at the lower concentrations and as the concentrations increase the curves become more erratic owing to the difficulty of detecting small changes in concentrated sugar solutions. A water control showed that the leakage of sucrose amounted to no more than 0.2 umoles per g fr wt over the period of time during which uptake was measured.

The best approximation of all of the curves seems to be a straight line.

Figure 4 shows the effect of sucrose concentration on sucrose uptake. The rate was arrived at by dividing the total uptake by the time period over which uptake was measured. As seen by the theoretical curve, the data agree very well with the typical Michaelis and Menten hyperbolic substrate concentration curve for which the constants were derived from a Lineweaver and Burk plot of the data.

Figure 2. Sucrose Uptake Vs Time. In these experiments 1 g of slices was incubated in water for 1 hr. This was followed by 2-10 ml rinses and then the uptake solution was added to the slices. Ten ml of uptake solution was added and the amount of sucrose removed in the sampling was taken into account when calculating the data. The first sample was taken 15 min after addition of the uptake solution (time zero on the graph). Samples of 0.5 ml were taken every 15 min. Each curve represents data from 1 day's experiment.


16
12
8
4
Sucrose uptake, umoles/g fr wt
0.01M
0.005M
0.001M
15
30
45
60
Time, min

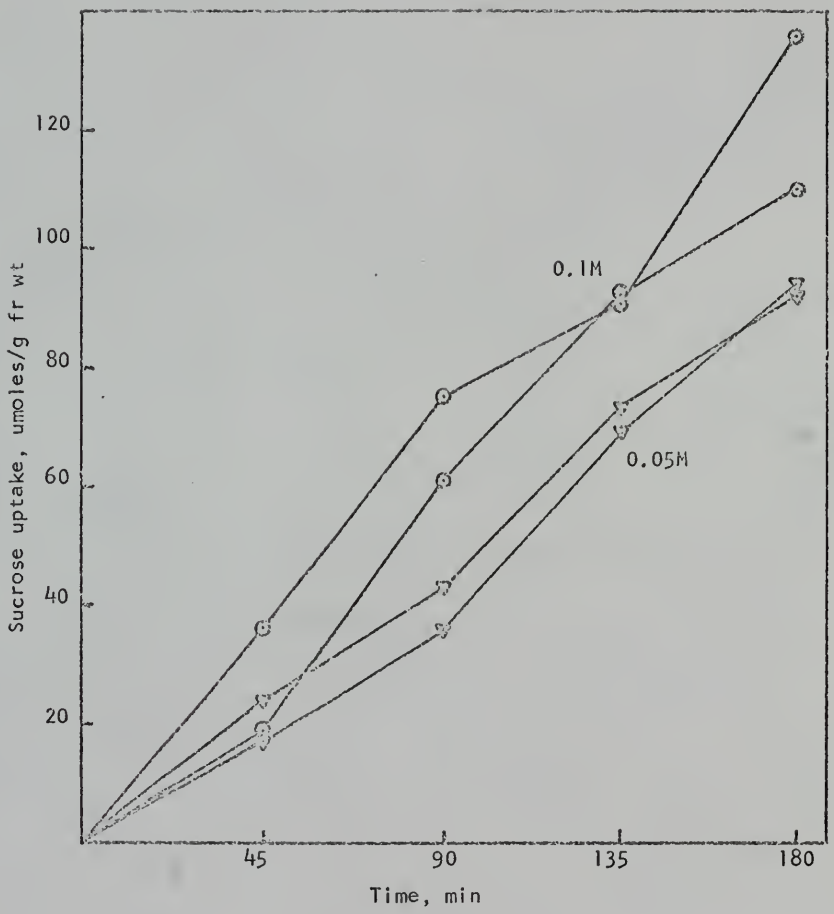


Figure 3. Sucrose Uptake Vs Time. Conditions were the same as in Figure 2. Samples were taken every 45 min.

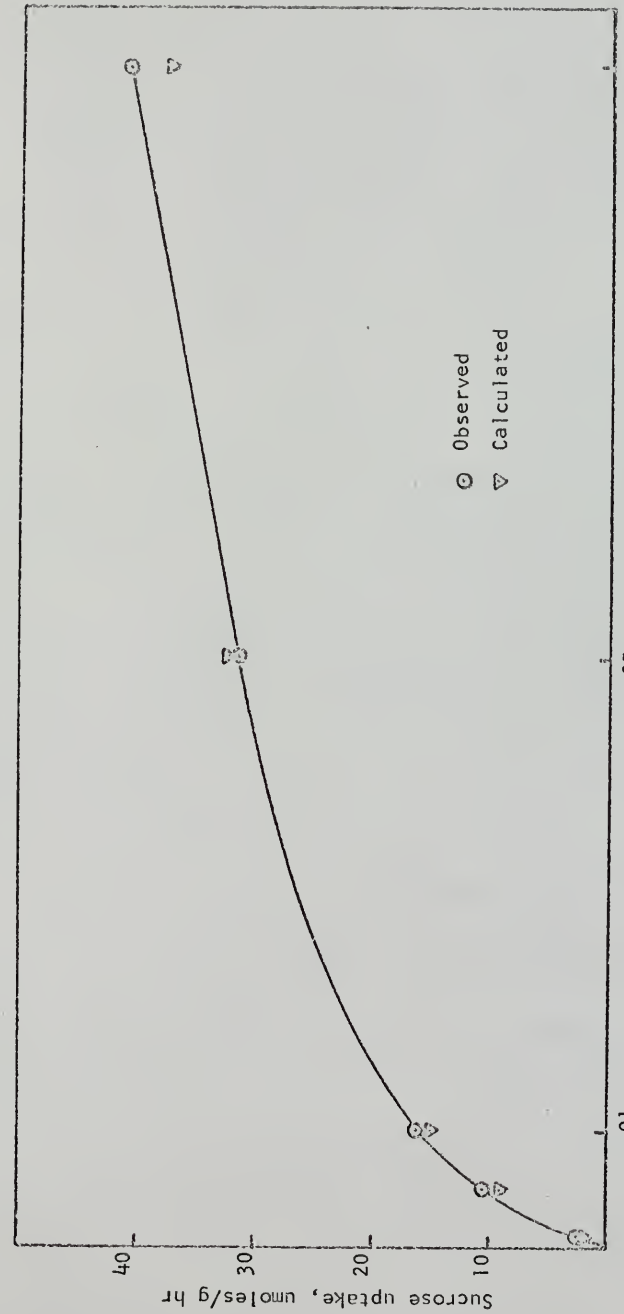


Figure 4. Sucrose Uptake Vs Concentration. The data presented are from the same experiments reported in Figures 2 and 3. Values of 45 umoles/hr and 0.02M were used for Vmax and Km to obtain the calculated points. The "observed" points are averages of 2 determinations.

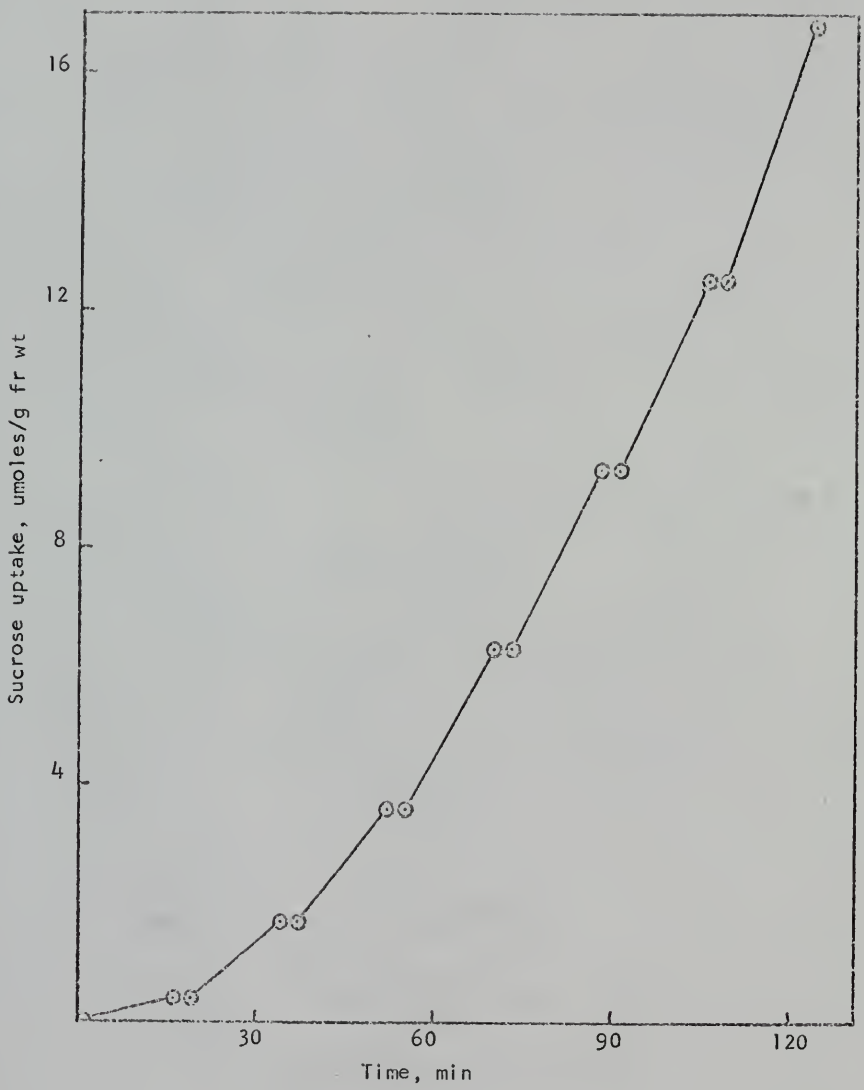
The experiment of Figure 5 shows sucrose uptake when the bathing solution is maintained at a constant sucrose concentration. In this experiment the volume of the bathing solution was reduced to 4 ml for greater accuracy. At the end of each 15-min uptake period the solution was removed and fresh 0.001M sucrose was added to the slices. The rate of uptake can be seen to increase with time over a period of 2 hr.

Figures 6 and 7 show the rate of glucose uptake with time. As with sucrose, the glucose uptake curves roughly represent straight lines with possibly a little more tendency for the rates to decrease with time. In the case of 0.005M glucose the concentration of the sugar solution was reduced by about half during the course of the experiment, yet the rate of uptake over the last three periods was about the same. Very similar data were collected using fructose but they are not presented.

Figure 8 shows the uptake vs concentration data for glucose and fructose. In the case of glucose and fructose the data do not fit at all when an attempt is made to find the constants Vmax and Km by plotting the data according to a Lineweaver and Burk plot. Neither do the data agree with what would be expected if diffusion were the driving force for glucose uptake.

Uranyl ion has been shown to inhibit sugar uptake (67) and to act at the cell surface (55). Uranyl ion caused a partial inhibition of glucose and fructose uptake. This was true both when uranyl ion was added to the uptake solution and when the slices were treated with uranyl ion prior to the uptake period. The dotted lines in Figure 9 show the uptake vs concentration data for glucose and fructose when uptake was measured following a pretreatment with uranyl ion. Table 2

Figure 5. Sucrose Uptake at Constant Sucrose Concentration. One g of slices was incubated in water for 1 hr followed by 2-10 ml rinses. 4.2 ml of 0.001M sucrose was then added and 1 min later a 0.2 ml sample was taken. A second sample was taken 15 min after the first. Then the entire solution was removed and 4.2 ml of a fresh 0.001M sucrose solution added. This procedure was continued throughout the experiment.


16
12
8
4
Sucrose uptake, umoles/g fr wt
30
60
90
120
Time, min

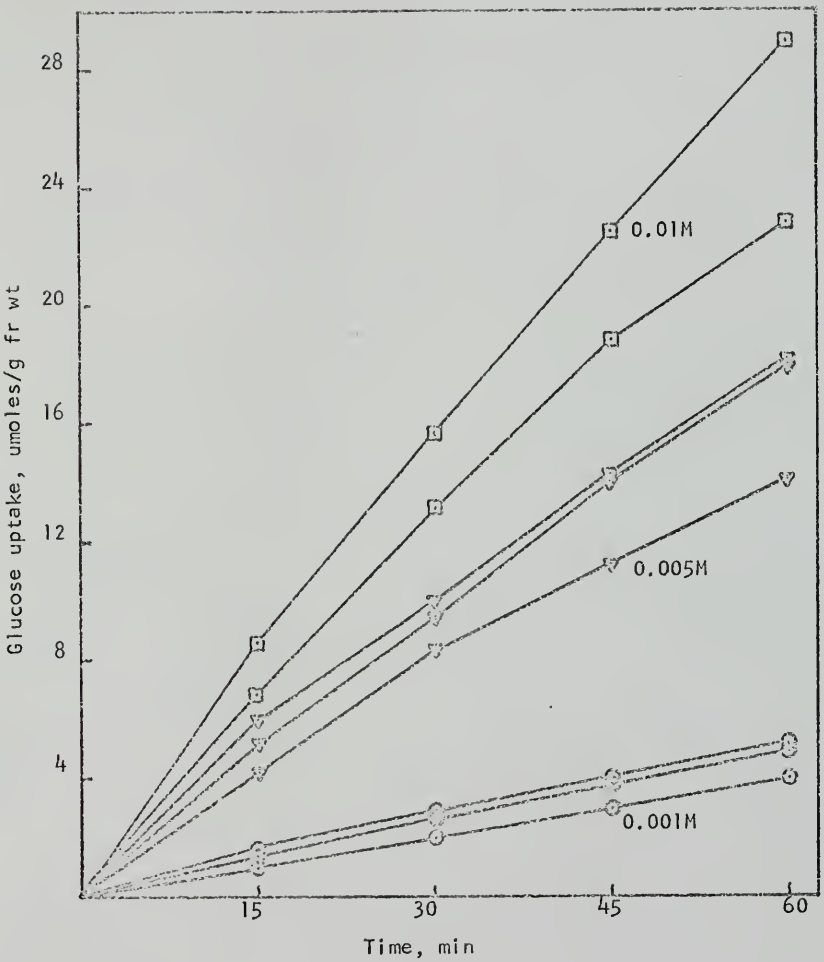


Figure 6. Glucose Uptake Vs Time. The data from which these curves were prepared come from the same type of experiments as shown in Figures 2 and 3 with the exception that glucose was the sugar taken up.

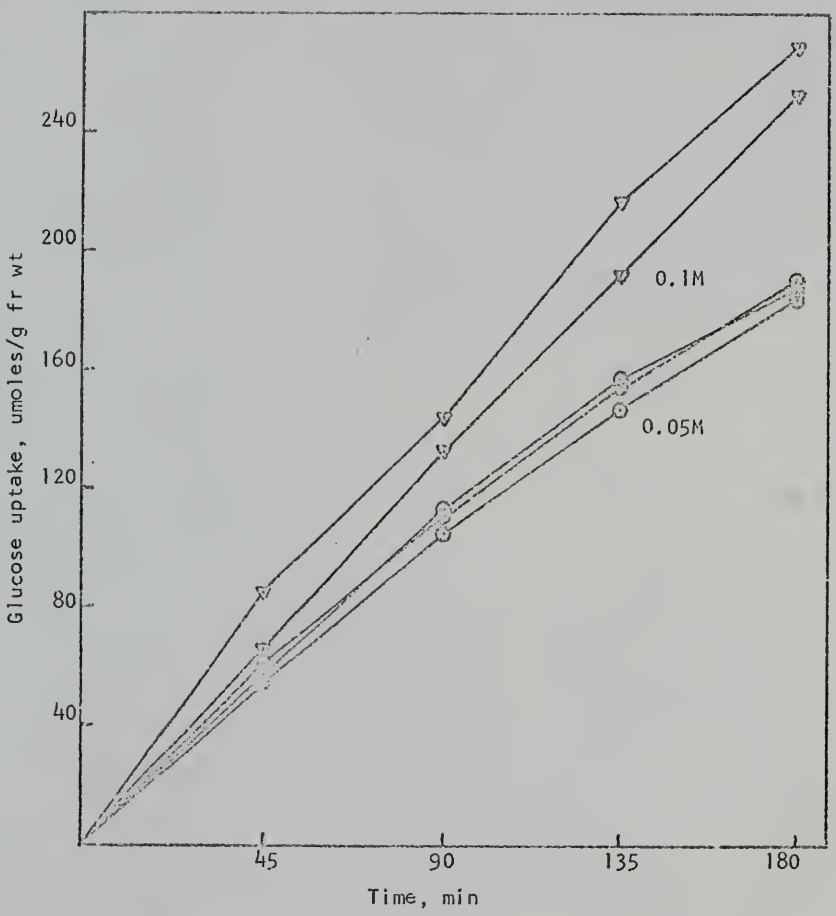


Figure 7. Glucose Uptake Vs Time. The data from which these curves were prepared come from the same type of experiments as shown in Figures 2 and 3 with the exception that glucose was the sugar taken up.

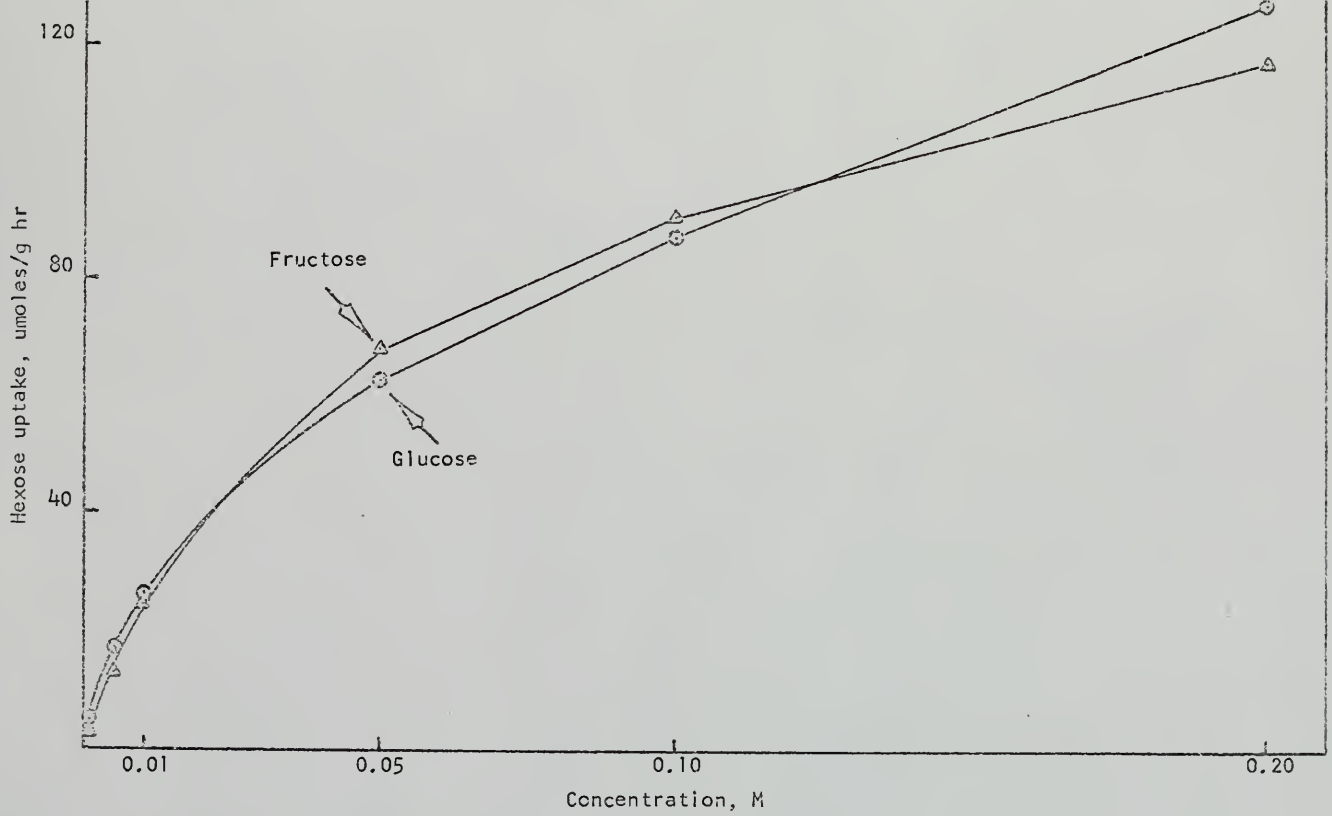


Figure 8. Rates of Fructose and Glucose Uptake Vs Concentration. The data presented are from the same experiments reported in Figures 6 and 7 and similar experiments using fructose.

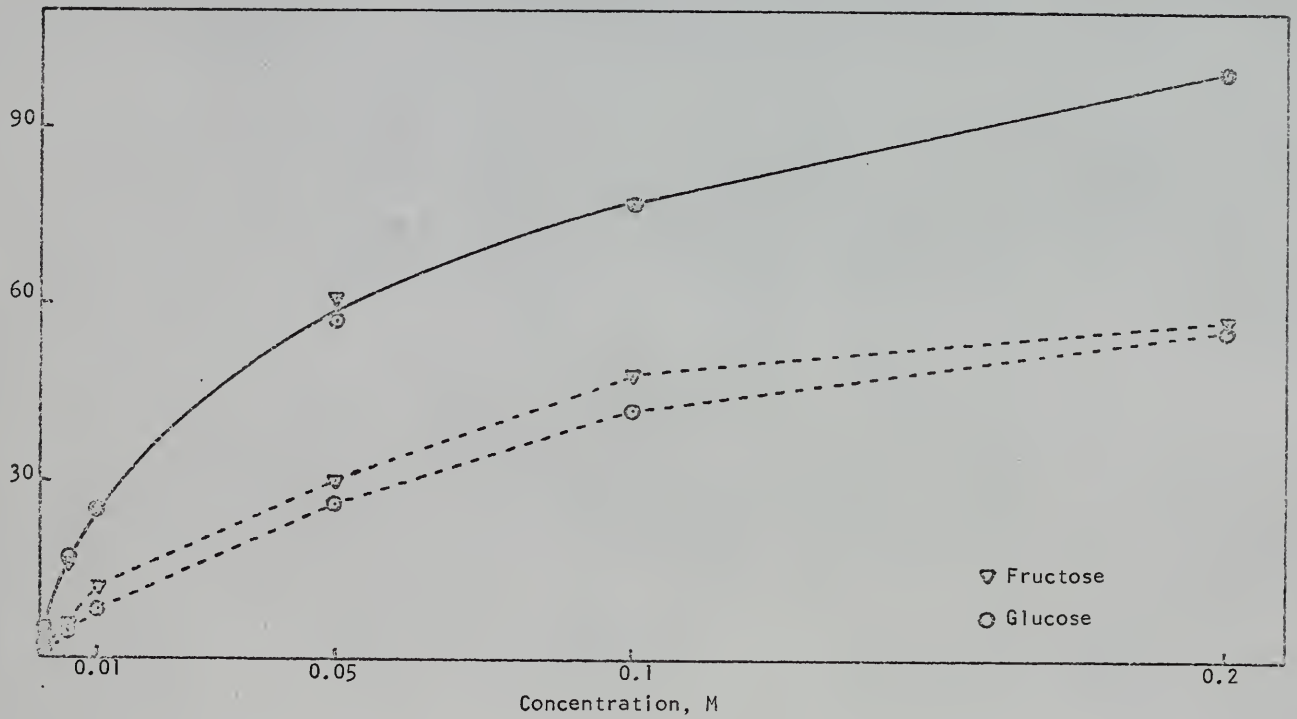


Figure 9. Glucose and Fructose Uptake as Affected by Uranyl Ion Pretreatment. One g of slices was placed in either water or uranyl nitrate for 1 hr. The slices were given 2 rinses, placed in water for 30 min, given 2 more rinses and finally placed in the sugar solutions. Two samples were taken, the first 15 min after the uptake solution was added and the second at the end of the uptake period. Uptake was measured over a period of 60 min with 0.001 and 0.005M sugar, 90 min with .01M sugar and 180 min with 0.05, 0.1, and 0.2M sugar. The data for glucose are an average of 2 determinations, the data for fructose are from 1 determination.

Table 2

Glucose Uptake as Affected by Uranyl Ion in the Bathing Solution

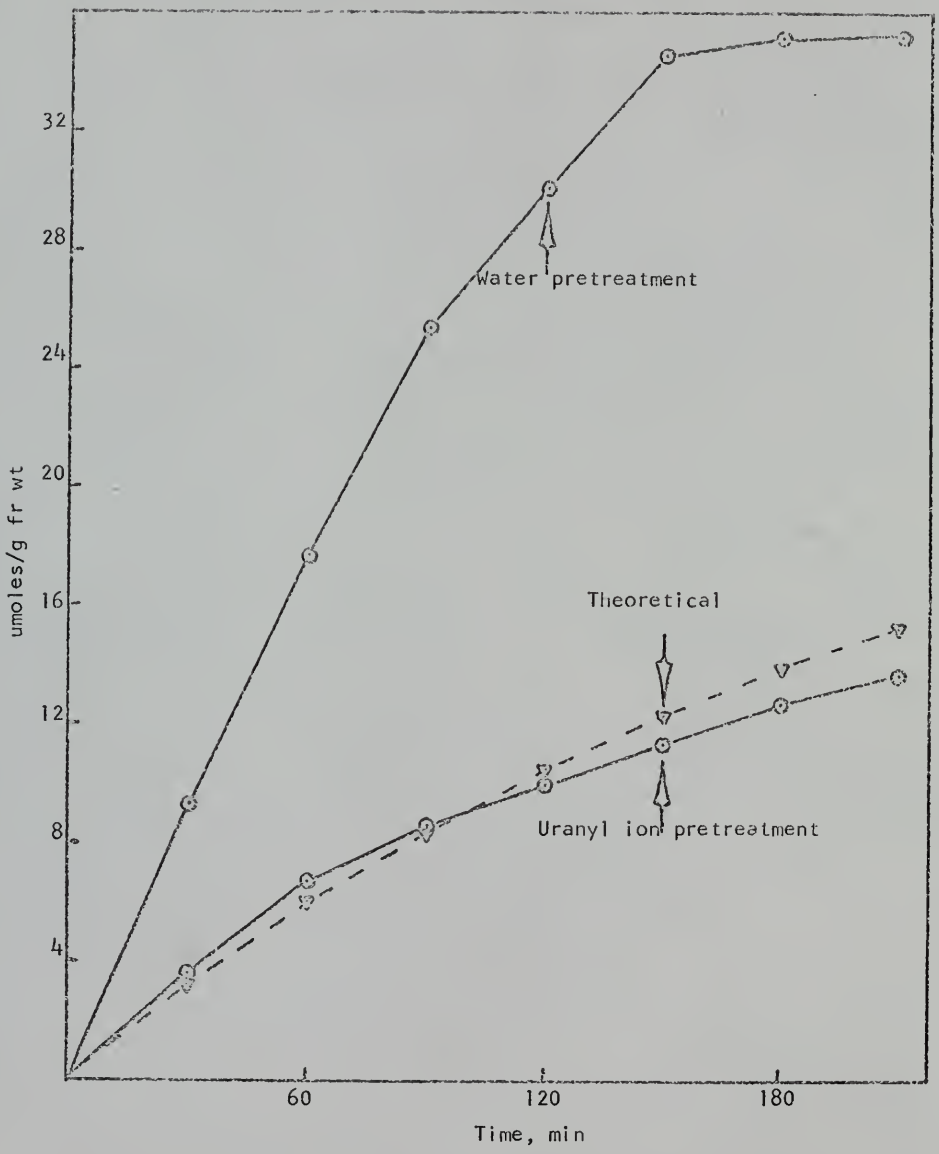
Glucose concentration M	Uptake period hr	Inhibition %
.001	1	58
.005	1	54
.005	2	53
.01	2	44
.01	3	45
.1	3	34

shows the effect of uranyl ion on glucose uptake when uranyl ion was added to the uptake solution. Notice that in both pretreatment and treatment during uptake, the inhibition was greater at lower concentrations of glucose. The inhibition was greater when the slices were pretreated with uranyl ion. This is perhaps a result of a long term effect of uranyl ion since in the pretreatment experiments uranyl ion was first applied to the slices 105 min prior to the uptake period.

The glucose uptake vs concentration curve after uranyl ion pretreatment resembled, at least at low concentrations, a diffusion curve (Figure 9). Since glucose is not accumulated by the tissue but is rapidly used for sucrose synthesis or is fermented (60, 65) the question arises as to whether the kinetics presented are those of uptake, i.e., that glucose is limited by diffusion, or those of the hexokinase reaction. Jones (83) studied the properties of hexokinase from the corn scutellum and reports the Km for glucose as 6.5×10^{-6}M. A Km of this magnitude indicates that diffusion and not the hexokinase reaction is limiting. If diffusion is the driving force for uptake and if it is assumed that the internal glucose concentration remains constant and very low then the uptake should be a straight line function of the glucose concentration. A straight line was, in fact, obtained at concentrations of 0.01M and below. The deviation from linearity at higher concentrations may be due to higher internal concentrations of glucose and to a saturation of the glucose utilization process at the higher concentrations.

To check this hypothesis further the rate of uptake into uranyl treated and untreated slices was followed with time (Figure 10). In the case of glucose alone the uptake proceeded at almost a constant rate until the glucose in the uptake solution had been depleted to a

Figure 10. Uranyl Ion Pretreatment and Glucose Uptake. The pretreatment consisted of 1 hr in either water or 0.003M uranyl nitrate. The slices were then given 2 rinses, 30 min in water, 2 more rinses, and then the 0.01M 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 curves are an average of 2 determinations. The volume of the uptake solution was 6 ml so that larger differences in Klett readings were noted over short time periods.


32
28
24
20
16
12
8
4
umoles/g fr wt
Water pretreatment
Theoretical
Uranyl ion pretreatment
60
120
180
Time, min

level below that of the detection system used for glucose analysis. This curve cannot be explained on the basis of diffusion alone. It might be argued that the rate of utilization of glucose by the tissue is constant and that the curve simply represents the glucose utilization rate. However, as shown elsewhere by both uptake and accumulation data (Figure 7, Table 9), the uptake mechanisms of the tissue are not saturated even at much higher hexose concentrations. In contrast the curve 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 (Figure 10).

It is postulated that glucose uptake is the total of two processes, one consisting of simple diffusion soon after uranyl ion treatment and one an active process which is subject to inhibition by uranyl ion binding.

If this is true it means that the active component as represented by the difference in the two curves in Figure 10 is increasing with time (i.e., since the external concentration is decreasing, diffusion is decreasing and the active component must be increasing in order to maintain a steady rate). With this in mind an experiment was run in which the concentration of glucose was kept constant by renewing the solution after each sample as was done with sucrose (Figure 5). The results of this experiment failed to support the hypothesis (Figure 11). Glucose uptake remained constant with time in spite of the fact that the concentration was kept constant. The uptake over a period of 2 hr was very close to the value obtained with declining glucose concentration. When uranyl nitrate was added to the uptake solution in this

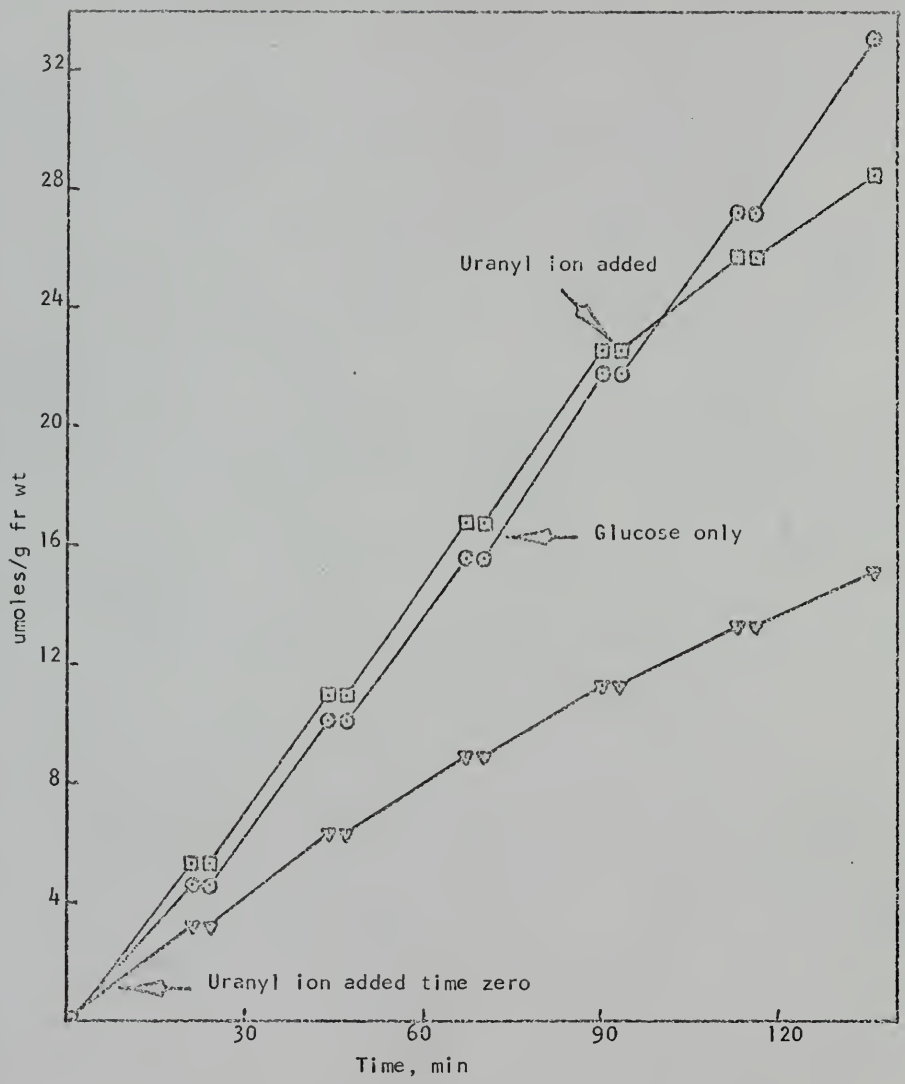


Figure 11. Glucose Uptake at Constant Concentration. Tissue was subjected to 1 hr in water, 2 rinses, and then the 0.005M uptake solutions. The first sample was taken 1 min after adding the solution. Twenty minutes later a second sample was taken and the uptake solution was withdrawn and fresh solution added. This procedure was continued throughout the experiment. Where indicated on the graph the solution contained 0.003M uranyl nitrate. The uptake volume was 4 ml.

experiment the rate of uptake declined with time. This may be due to a long term effect of uranyl ion. The amount of inhibition caused by uranyl ion was the same whether it was added at the beginning of the uptake sampling or added after the tissue had been taking up glucose for 80 min.

An experiment was run to determine whether or not the inhibition of glucose uptake by uranyl ion was parallelled by an inhibition in the amount of sucrose gained by the tissue. The slices were placed in water or 0.003M uranyl nitrate for 1 hr, rinsed, placed in water for 30 min, rinsed, and placed in .1M fructose or glucose for 3 hr after which the slices were rinsed and killed for analysis of tissue sucrose. A control group was killed after the 30 min water incubation. The net increase in tissue sucrose in the water treated groups was 70 and 73 umoles in fructose and glucose, respectively, and the inhibition by uranyl ion was 54 and 53%, respectively. Uptake (Figure 9) was inhibited 38 and 45%.

Sucrose Uptake

In order to study sucrose uptake it should be established that sucrose as such is the sugar being taken up. Several lines of evidence will be presented to show that sucrose is taken up without inversion by slices of the corn scutellum. The effects of several inhibitors on sugar uptake will also be presented in this section.

The amount of inversion of sucrose in the external solution is small as measured by the appearance of glucose in the solution. For instance at a sucrose concentration of 0.05M the maximum amount of glucose noted in the solution was 0.0006M. The amount varied over the uptake period from this value to an amount below the limit of detection

for the amount of sample used. This would mean a maximum hexose concentration of 0.0012M and an uptake according to Figure 8 of approximately 7 umoles of hexose or 3-1/2 umoles of sucrose per hr which is roughly one-tenth of the rate of sucrose uptake observed (Figure 4). This argument implies the assumption, justified by Figure 8, that the two hexoses are taken up at about the same rate. This argument has been presented before by Humphreys and Garrard (64). The possibility exists that inversion takes place in a position such that hexoses move preferentially toward the point of uptake.

Kinetic data on the uptake of sucrose, glucose, and fructose were presented in the first section. The overall shapes of the uptake rate vs concentration curves is considerably different. Whereas hexose uptake increases with concentration over the range shown, sucrose uptake approaches a maximum. The considerable difference in the effect of concentration on uptake is an indication that two different processes are at work.

By comparing the data in Figures 4 and 8 it can be seen that the total amount of carbon taken up from 0.1M solution is higher when hexoses are supplied. In experiments measuring tissue sucrose it was noted that the amount of sucrose gained by the tissue was the same or higher when the slices were incubated in sucrose as compared to incubation in hexose. In order to check this effect on the same group of slices an experiment was run in which uptake was allowed to proceed over a period of 205 min from solutions of 0.1M sucrose or glucose. Duplicate flasks were run. Uptake was measured over 3 hr of the uptake period. The amount of carbon taken up from glucose solution was 110% of that taken up from sucrose solution although the amount of sucrose

gained by the slices was the same in both cases. The slices were incubated in water following the uptake period and then rinsed, killed and analyzed for sucrose content.

When gas exchange studies were carried out using 300 mg of tissue in the Warburg flasks it was found that the addition of sucrose, glucose, or fructose to the solution caused high rates of fermentation to occur. It was assumed that the amount of O_2 consumption represented sugars being completely respired whereas the amount of CO_2 evolved in excess of the amount of O_2 consumption represented sugars being fermented. It has been demonstrated previously that under similar conditions ethanol is produced in amounts equal to the excess CO_2 (65).

The data in Table 3 show the amount of fermentation over a 2 hr period caused by the various sugar solutions. In the fermentation experiments the slices were incubated in water for 1 hr in the water bath, blotted, and weighed into Warburg flasks which contained the sugar solutions. The flasks were then attached to the manometers, equilibrated for 15 min and the readings begun. No effort was made to regulate the time between adding the slices to the sugar solutions and the beginning of the readings.

The rate of respiration was reasonably constant regardless of the solution. The rate of fermentation was higher in fructose or the hexose combination than it was in sucrose. In terms of hexose uptake, the rate of sucrose uptake at 0.01M is 35 umoles of hexose equivalent per hr compared to a rate of 25 umoles for hexose. Uptake of glucose and sucrose from 0.01M solution by the same day's slices gave the following results; sucrose 17 umoles/hr (or 34 umoles hexose), glucose 28 umoles/hr. If the amount of fermentation is a function of the con-

Table 3

Fermentation in Water and Sugar Solutions

Solution	Respiration umoles hexose/hr	Fermentation umoles hexose/hr	No. of experiments
(Data averaged from several day's preparations)			
Water	5.8 (0.4)*	4.3 (0.8)	5
0.01M sucrose	4.9 (0.4)	11.6 (1.9)	5
0.01M fructose	4.9 (0.2)	12.0 (1.7)	4
0.005M fructose & 0.005M glucose	5.3 (0.1)	13.8 (1.2)	2
0.1M sucrose	5.9	28.5	1
0.1M fructose	5.0 (0.1)	36.8 (1.8)	2
0.1M glucose	4.8	40.5	1

*Numbers in parentheses indicate the average deviation. See text, and materials and methods, for experimental detail.

centration of hexose in the fermentation compartment and if sucrose is being inverted, even in the process of uptake, the rate of fermentation in sucrose should be higher than it is. The same argument can be made in the case of 0.1M sugar, since the amount of fermentation in hexose solution is proportionately greater than the amount of hexose uptake.

Further evidence that sucrose is taken up without inversion comes from the effects of uranyl ion on sugar uptake. The effect of uranyl nitrate pretreatment on the uptake of hexoses has already been given. Table 4 shows the effect of uranyl ion on sucrose uptake. Uranyl ion more completely inhibits the uptake of sucrose. With hexose the effect is to cut the uptake roughly in half. If sucrose were being inverted prior to uptake, the expected effect of uranyl ion on the basis of the hexose uptake after uranyl treatment curves would be to cut the uptake of sucrose in half. It might be argued that uranyl ion is inhibiting the inversion of sucrose but the inversion of sucrose as measured by the amount of glucose found in solution is higher after uranyl ion pretreatment than it is after water pretreatment. In measuring sucrose, it was consistently noted that the non-invertase-treated sample gave slightly higher Klett readings in the cases where the slices had been pretreated with uranyl ion.

The effects of anerobic conditions on the uptake of sucrose, glucose and fructose were determined by incubating slices in water for 1 hr and then transferring the slices to sugar solutions and incubating in air or under nitrogen. Nitrogen was continuously bubbled through the solution. Sucrose uptake in N_2 was only 33% of that in air whereas with glucose and fructose the rates of uptake in N_2 were 65% of those in air.

Table 4

The Effect of Uranyl Nitrate Pretreatment on Sucrose Uptake

Concentration M	Uptake umoles/hr	
	Water pretreatment	Uranyl nitrate pretreatment
0.001	4.3	0.3
0.005	14.3	2.7
0.01	21.0	5.4
0.05	29.0	5.7
0.1	41.0	2.3

Slices (1.0 g fr wt) were placed in either water or uranyl nitrate for 1 hr. The slices were given 2 water rinses, placed in water for 30 min, given 2 more water rinses 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 a period of 60 min with 0.001M and 0.005M sucrose, 90 min with 0.01M sucrose and 180 min with 0.05 and 0.1M sucrose. Rates of uptake in the first three are averages of the results of two experiments, the last two are from one experiment.

Table 5 shows the inhibition of sugar uptake by phloridzin. At 1×10^{-3}M this inhibitor caused about twice the amount of inhibition of sucrose uptake as it did with glucose uptake. With neither sugar was uptake inhibited strongly by the low concentrations used with animal systems.

Dinitrophenol (DNP) also inhibited sugar uptake (Table 6). The inhibition was considerably greater with sucrose than with glucose; the sucrose inhibition approached 100% whereas the inhibition of glucose uptake approached 50%. Sucrose uptake in the presence of 1×10^{-4}M DNP was linear with time for at least 1 hr.

The uptake of sucrose was measured in the presence of several disaccharides to determine if the uptake process was subject to inhibition by molecules of similar structure. Several of the disaccharides caused an increase in the non-invertase-treated samples. This caused confusion in interpreting the results since it was not known whether the increased amount of glucose in solution was coming from hydrolysis of the disaccharide or from an increased hydrolysis of sucrose caused by the presence of the disaccharide. Unless otherwise stated these experiments were run by incubating first for 1 hr in water and then placing the slices in 0.01M sucrose plus the various disaccharides in concentrations of 0.005, 0.01, and 0.05M. The uptake period was 1 hr.

Lactose was not taken up by the tissue as determined by measuring the lactose concentration before and after 1 hr incubations by the reducing sugar method. When solutions were tested for glucose the amount was found to be insignificant. When sucrose uptake was measured in the presence of lactose there was an inhibition of 33% in the case of 0.05M lactose if it is assumed that the higher glucose comes from

Table 5

Inhibition of Sugar Uptake by Phloridzin

Concentration of phloridzin M	Inhibition %	
	Glucose	Sucrose
1×10^{-5}	0	-
1×10^{-4}	11	0
5×10^{-4}	-	14
1×10^{-3}	13	25
2×10^{-3}	-	47
3×10^{-3}	-	60

The slices (1.0 g fr wt) were incubated in water for 1 hr, rinsed twice, and placed in a solution of 0.01M sugar and phloridzin as indicated. Results are based on a non-inhibited control. Uptake was measured over a 2 hr period.

Table 6

The Inhibition by DNP of Uptake from 0.01M Sugar Solution

Concentration of DNP	Inhibition %	
	Glucose	Sucrose
1×10^{-5}M	2	31
3×10^{-5}M	14	51
1×10^{-4}M	35	89
3×10^{-4}M	43	94

The schedule was the same as with phloridzin. The uptake period was 90 min.

the increased hydrolysis of sucrose in the presence of lactose. In other words, the same amount of sucrose was found at the end of the uptake period with or without lactose. There was more glucose in solution at the end of the uptake period when using sucrose and lactose than there was in the case of lactose or sucrose alone. If this glucose came from hydrolysis of sucrose then uptake was inhibited. This is a reasonable assumption since no hydrolysis was found in the case of lactose whereas an increase in sucrose hydrolysis in the presence of various inhibitors was commonly found.

Sucrose uptake was not inhibited by melibiose, trehalose or cellibiose.

When maltose was supplied to the tissue there was considerable hydrolysis. As shown in Table 7 the glucose concentration increased from the beginning of the uptake period to the end, while the concentration of maltose declined. The amount of uptake is expressed in terms of umoles of glucose. The concentration of glucose is insufficient to account for the uptake (Figure 8) indicating that some maltose was taken up without hydrolysis. This experiment shows firstly that the disaccharide is being hydrolyzed and secondly that following hydrolysis the monosaccharide can be detected in the external solution. It might be pointed out that maltose would be an expected product of the breakdown of starch in the endosperm.

The appearance of glucose in solution coming from both maltose and sucrose makes it difficult to determine the effect of maltose on sucrose uptake.

When turanose was added to the slices no glucose appeared in solution. When turanose and sucrose together were added to the slices the

Table 7

Maltose Uptake

Concentration of added maltose	Change in glucose umoles/flask	Change in maltose umoles/flask	Uptake umoles glucose/hr
0.005M	6 to 24	39 to 23	14
0.01M	10 to 40	80 to 52	26
0.05M	22 to 78	346 to 274	88

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 1 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. The results are averages of 3 determinations on 2 days' slices.

glucose readings were little or no higher than when sucrose was added alone. Turanose at 0.05M caused approximately a 30% inhibition of sucrose uptake (Table 8). The uptake of turanose alone was checked by using the reducing sugar method and was found not to be taken up by the slices. That the effect of turanose is not an osmotic effect was demonstrated by measuring the uptake of sucrose alone, sucrose in the presence of turanose and sucrose in the presence of mannitol.

Several experiments were designed to show the effect of tissue sucrose level on the rate of sucrose uptake. The experiments are summarized in Table 9. Generally there is an inverse correlation between the amount of sucrose in the tissue and the rate of uptake. The tissue was subjected to various treatment sequences so as to vary the amount of sucrose in the tissue prior to measuring uptake. In those three cases marked by an asterisk the sequences were the same except for the concentration of sugar during the first 3 hr and in each case sucrose uptake was measured during the fifth hr from the time the experiment started. In these cases there is an excellent inverse correlation between the amount of tissue sucrose stored and the rate of uptake. This is to be expected since there must be some limit to the amount of sucrose that can be accumulated by the slices and one would expect the rate of uptake to be reduced as this limit is reached.

Gas Exchange

When gas exchange experiments were carried out using 300 mg of tissue in the Warburg flasks the amount of O_2 taken up by the tissue was limited by the rate at which O_2 diffuses into water. This is shown by the following: (a) The rates of O_2 consumption noted in these experiments were equal to the limits of the rate at which O_2 diffuses into

Table 8

The Effects of Turanose and Mannitol on Sucrose Uptake

	Uptake umoles/g hr		
Experiment	0.01M sucrose	0.01M sucrose & 0.05M turanose	0.01M sucrose & 0.05M 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 over 1 hr in experiment 1 and 2 hr in experiments 2 and 3.

Table 9

Sucrose Tissue Level and Sucrose Uptake

Treatment sequence	Tissue level umoles/g	Sucrose uptake umoles/g hr
Water 3 hr, rinse, water 1 hr, rinse, sucrose 1 hr	50	25*
Water 1 hr, rinse, sucrose 1 hr	68	17
0.02M fructose 3 hr, rinse, water 1 hr, rinse, sucrose 1 hr	81	21*
0.1M fructose 3 hr, rinse, water 3 hr, rinse, sucrose 1 hr	94	18
0.1M fructose 3 hr, rinse, water 1 hr, rinse, sucrose 1 hr	125	15*
0.1M fructose 3 hr, rinse, sucrose 1 hr	133	11

The results from 3 crops of seedlings are reported here. In each case the uptake period was 1 hr, the sucrose from which uptake was measured was 0.01M 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.

water (74). (b) When DNP was added under these conditions the amount of CO_2 evolution was increased; however, the amount of O_2 consumption was not. (c) When experiments were run using 100 mg of tissue in water the O_2 consumption increased on a per weight basis and the RQ decreased. The figures from two typical experiments were: with 300 mg tissue, 33 umoles O_2/g hr, RQ 1.3; with 100 mg tissue, 53 umoles O_2/g hr, RQ 0.8. Since when using 100 mg of tissue, the rate of O_2 consumption is well below the limit and since the RQ is quite low it was assumed that O_2 was not limiting under these conditions.

It was not known whether or not O_2 consumption was limited under conditions which prevailed in the water bath. An experiment in which sucrose uptake from 0.005M sucrose was measured as a function of the surface area of the liquid per weight of tissue showed that uptake was not limited by the amount of surface area. In this experiment uptake by 0.2, 0.4, and 1.0 g of tissue was measured. The volume to tissue ratio was kept the same by starting with 2, 4, and 10 ml of sucrose solution, respectively, and removing relatively the same amount of sample for sucrose analysis. The rates of uptake were 11.9, 11.4, and 11.3 umoles/g hr by 0.2, 0.4, and 1.0 g of tissue. These differences are too small to be considered significant and it was concluded that sucrose uptake was not limited by O_2 availability.

Experiments were also designed to show whether or not accumulation of sucrose by the tissue was affected by O_2 availability. In these experiments tissue sucrose was analyzed after placing 1 g of tissue in 10 ml of bathing solution as opposed to placing 1 g of tissue in 4 separate flasks each of which contained 2.5 ml of bathing solution. Where 0.1M sucrose was the uptake solution, analysis showed 110 umoles/g

where 1 flask was used and 139 umoles/g where 4 were used. (These figures are not net sucrose accumulated since the amount of tissue sucrose at the beginning of the uptake period has not been subtracted.)

Work by Humphreys and Garrard (70) and by the author have shown that the sucrose content of scutellum slices is reduced when slices are incubated in water indicating that sugar is the substrate for respiration. This was also noted when 0.25 g of tissue per flask was used. However, gas exchange studies using 100 mg of tissue have indicated an RQ in water substantially less than 1.0 indicating a substrate other than sugar. This inconsistency might be explained by the conversion of sucrose to organic acids.

Figure 12 shows the amount of fermentation in water and in two concentrations of sucrose when 300 mg of tissue was used. It is assumed in presenting these figures that for each umole of CO_2 evolved in excess of O_2 taken up one-half umole of hexose was being fermented. Figure 12B represents the same experiment but the amount of fermentation in the water control has been subtracted from that caused by sucrose.

On the basis of uptake experiments it was calculated that the solutions in these flasks would be depleted of sucrose after 3 hr in the case of 0.001M and 4 hr in the case of 0.005M sucrose. In the case of 0.001M sucrose the sugar-caused fermentation starts when the sugar is added, increases in rate, and then decreases at the time when the sugar should all have been taken up. The amount of fermentation caused by 0.005M sucrose is about three times that caused by 0.001M sucrose and as shown earlier in Figure 4 the rate of uptake is about three times as great. With 0.005M sucrose the agreement in timing between uptake and fermentation is not as close, however, the rate does begin to decline at a time when uptake should be complete.

Figure 12. Fermentation in Water and Sucrose. Each flask contained 300 mg of tissue. The sucrose was placed in the side arm and added at 30 min tc give the concentration indicated on the graph. Three sets of flasks were used, one each for the 2 concentrations of sucrose and one containing water. The values were obtained from; $CO_2 - O_2/2$ (in umoles). In B the values found with water have been subtracted from the values found with sucrose.

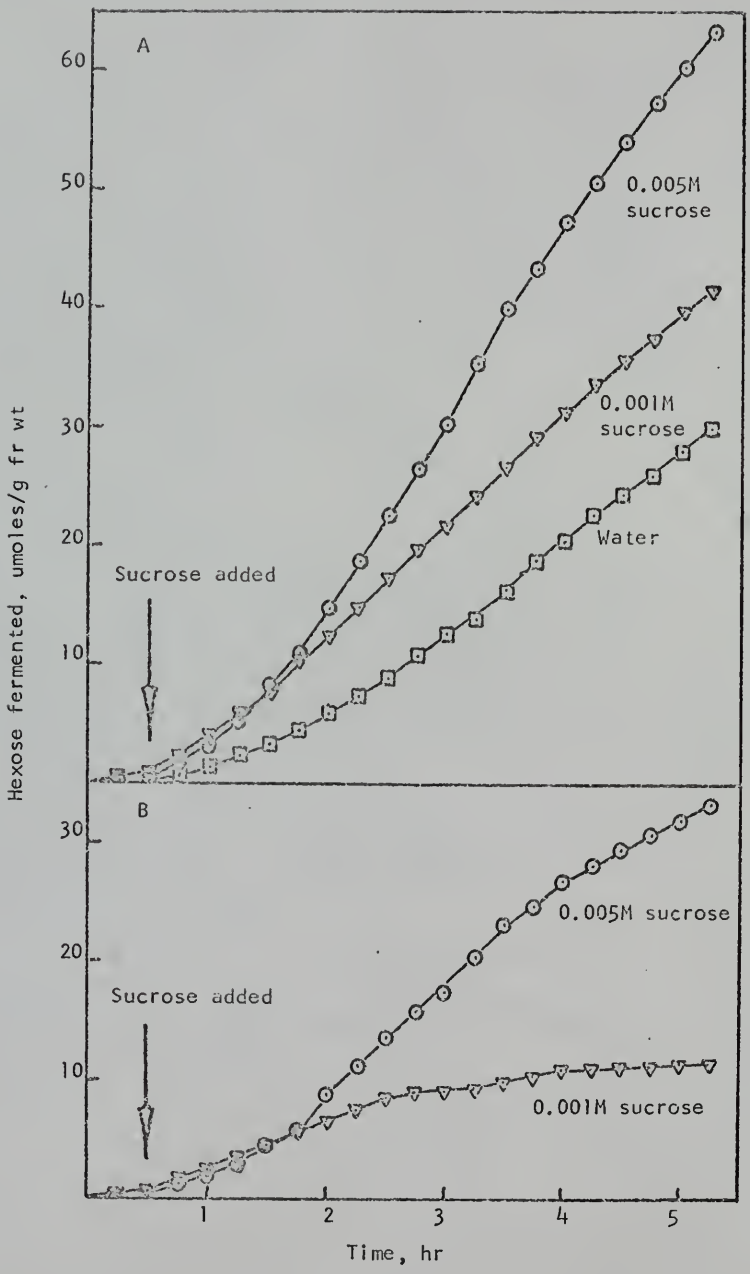

A
B
60
50
40
30
20
10
30
20
10
Hexose fermented, umoles/g fr wt
0.005M sucrose
0.001M sucrose
Water
Sucrose added
Sucrose added
0.005M sucrose
0.001M sucrose
1
2
3
4
5
Time, hr

Figure 13 shows the gas exchange in 0.1M sucrose as compared to that in water. These experiments were run under conditions in which O_2 was not limiting. With water the RQ is less than 1.0 and both O_2 uptake and CO_2 evolution proceed at constant rates throughout the experiment. When sucrose is added there is some depression in the rate of O_2 consumption. The rate of evolution of CO_2 on the other hand continues to increase throughout the experiment. Over the period of this experiment the uptake of sucrose would proceed at a constant rate (Figures 2 and 3). When 0.01M sucrose was added in the same type of experiment (data not presented) the pattern of gas exchange was the same; however, the O_2 consumption was not depressed as much and the rate of CO_2 evolution was not as high.

Figure 14 shows the same type of data for 0.1M glucose. Again the rate of O_2 consumption remains at a fairly constant rate while the evolution of CO_2 is greatly increased by the addition of glucose to the slices. The RQ during the last period measured was 2.3. As with sucrose, 0.01M glucose (data not presented) caused roughly the same pattern as 0.1M glucose and uptake proceeded at a constant rate (Figures 6 and 7).

If the assumption is made that the difference in CO_2 evolution in water versus that in sugar is due entirely to fermentation (65) then it is possible to calculate the number of umoles of hexose being fermented. Figure 15 shows the results of such a calculation. These results are from the same experiments as reported in Figures 13 and 14.

Rates of fermentation as suggested by the above data are consistant with data from experiments in which sucrose accumulation was measured. As pointed out earlier, the rate of fermentation is higher under conditions of limited O_2 in the Warburg apparatus and the amount of sucrose

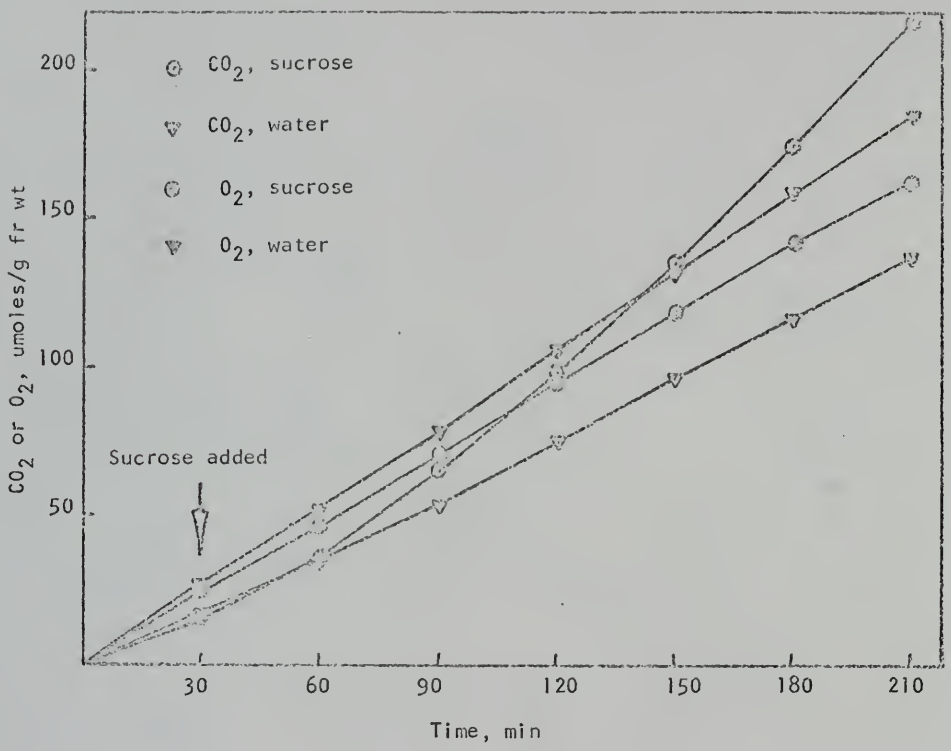


Figure 13. Gas Exchange in Water and 0.1M Sucrose. Experiments were run with 100 mg tissue per flask. The sucrose solution (1.0M) was added from the side arm 30 min after readings were begun.

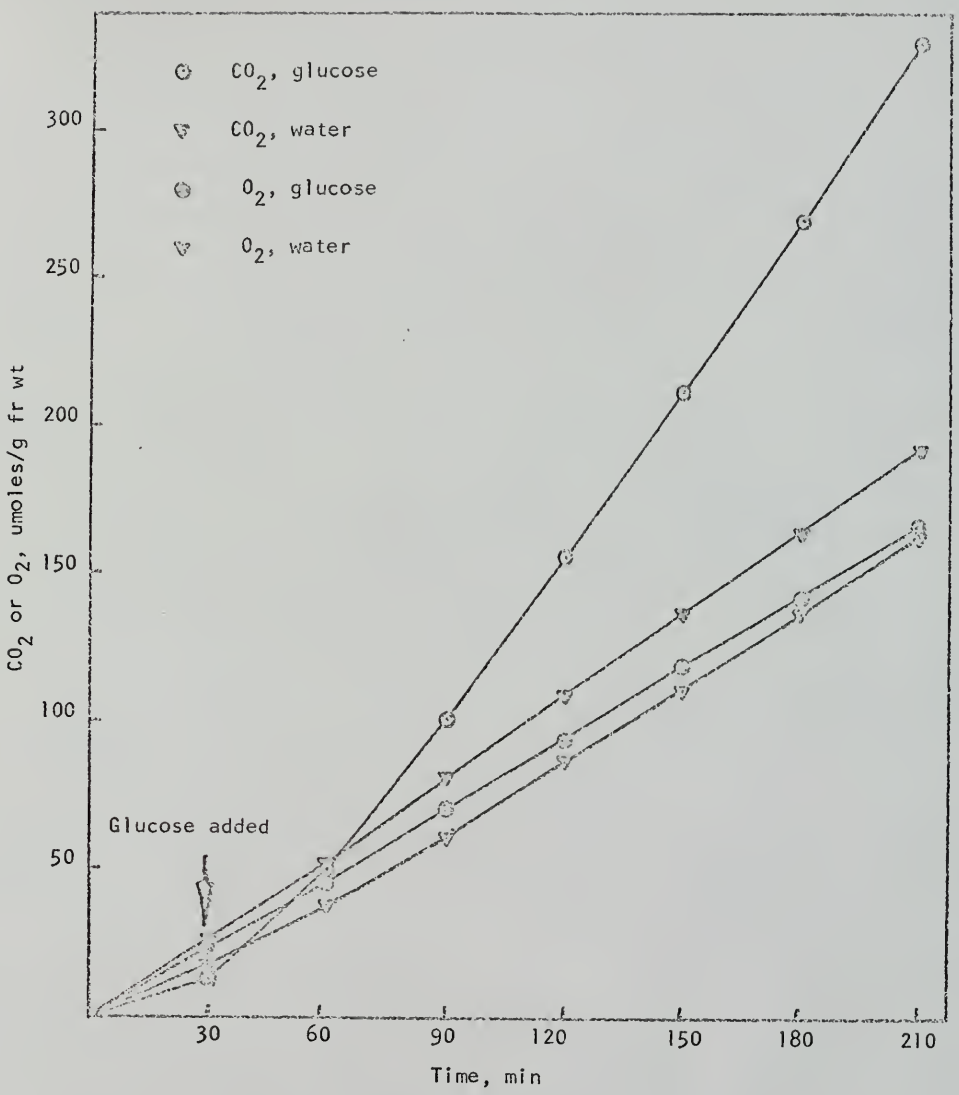


Figure 14. Gas Exchange in Water and 0.1M Glucose. Conditions were the same as in Figure 13.

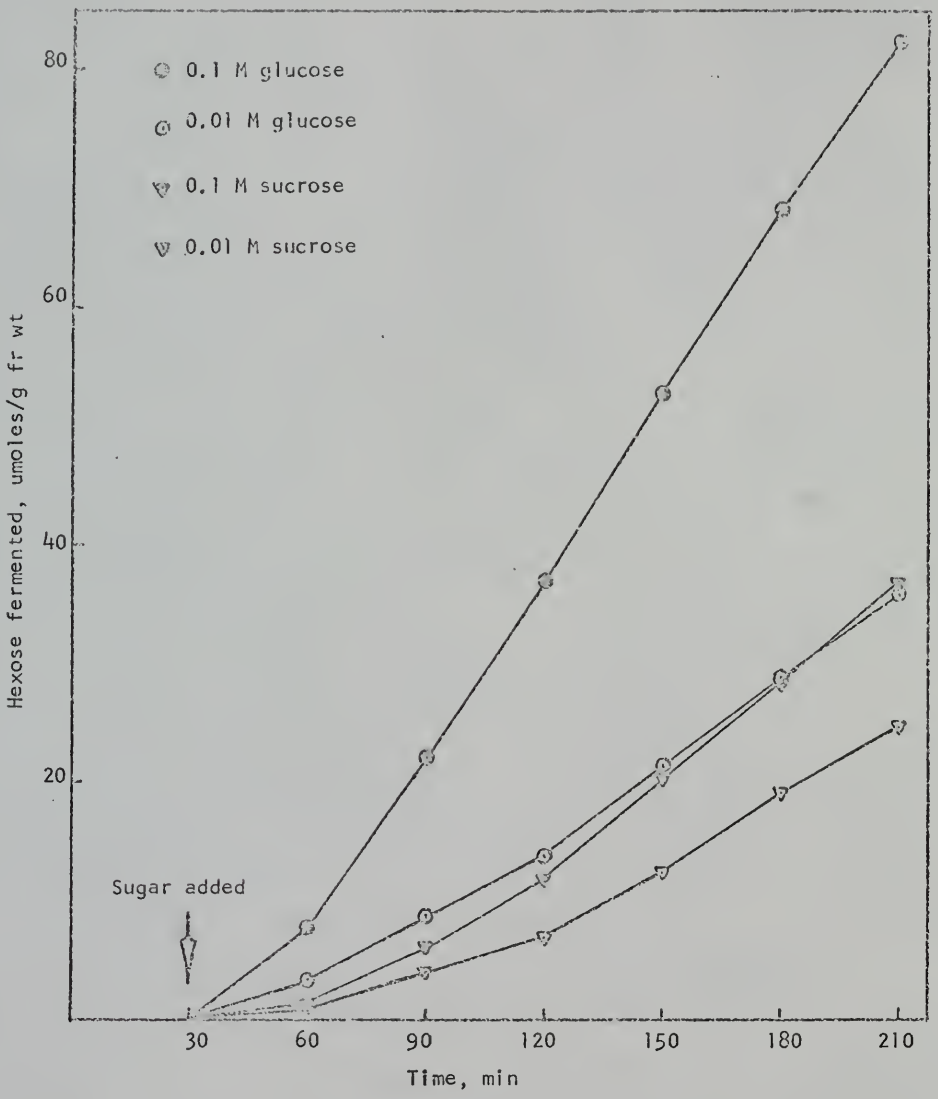


Figure 15. Fermentation in Sugar Solution. Conditions as in Figures 13 and 14. Results were calculated as CO_2 (in sugar sol)-CO_2 (in water)/2 (in umoles).

accumulated is lower under conditions of limited O_2 in the water bath. The rate of glucose uptake from 0.1M glucose was about 87 umoles/g hr. The rate of fermentation as indicated in Figure 15 was 28 umoles hexose/g hr. This would leave about 60 umoles of hexose available for accumulation, or enough to accumulate sucrose at a rate of 30 umoles/g hr. An experiment was run in which slices were incubated in 0.1M glucose for 3 hr. Each flask contained 250 mg of slices in 2.5 ml glucose solution. Sucrose accumulated at a rate of 35 umoles sucrose/g hr.

Metal Binding

Experiments were designed to determine the amount of uranyl ion that would bind to the slices, the effect of other cations on sugar uptake, and the relationship between the binding of some other cations and the binding of uranyl ion.

Table 10 shows the results of two experiments on the effect of cations on sucrose uptake. In the first experiment the cations were added to the uptake solutions. In the second experiment the slices were first treated with 0.01N HCl to remove cations attached to the slices, then treated with metal cations, rinsed, and placed in sucrose.

Uranyl ion reduces sucrose uptake when it is used as a pretreatment or when it is present in the uptake solution. Cations other than uranyl ion have little effect on uptake. Notice that the control, third column of Table 10, was treated with acid showing that the acid treatment does not seriously impair uptake.

The next series of experiments were designed to determine the quantities of the various ions that would bind to the surface of the slices. Figure 16 shows the amounts of the various ions that can be removed with 0.01N HCl after pretreatment with the various metals. Several

Table 10

Sucrose Uptake as Affected by Various Cations

Treatment	Uptake in umoles/g hr	
	With uptake solution	Pretreatment
Control	11.6	9.0
$CaCl_2$	11.5	11.0
$CoCl_2$	9.1	10.8
$MnCl_2$	12.1	10.1
$MgCl_2$	10.1	10.0
$AlCl_3$	9.7	9.5
$UO_2(NO_3)_2$	2.3	2.6

The data in the second column are from an experiment in which slices were incubated in water for 1 hr, rinsed with water, and placed in solutions containing 0.005M sucrose and 0.003M cation solution. The third column represents a 15-min incubation in 0.01N HCl followed by 2 rinses, 50 min in 0.003M cation solution, another rinse and finally the 0.005M sucrose solution from which uptake was measured. In both experiments the uptake period was 2 hr by 1 g of slices per flask.

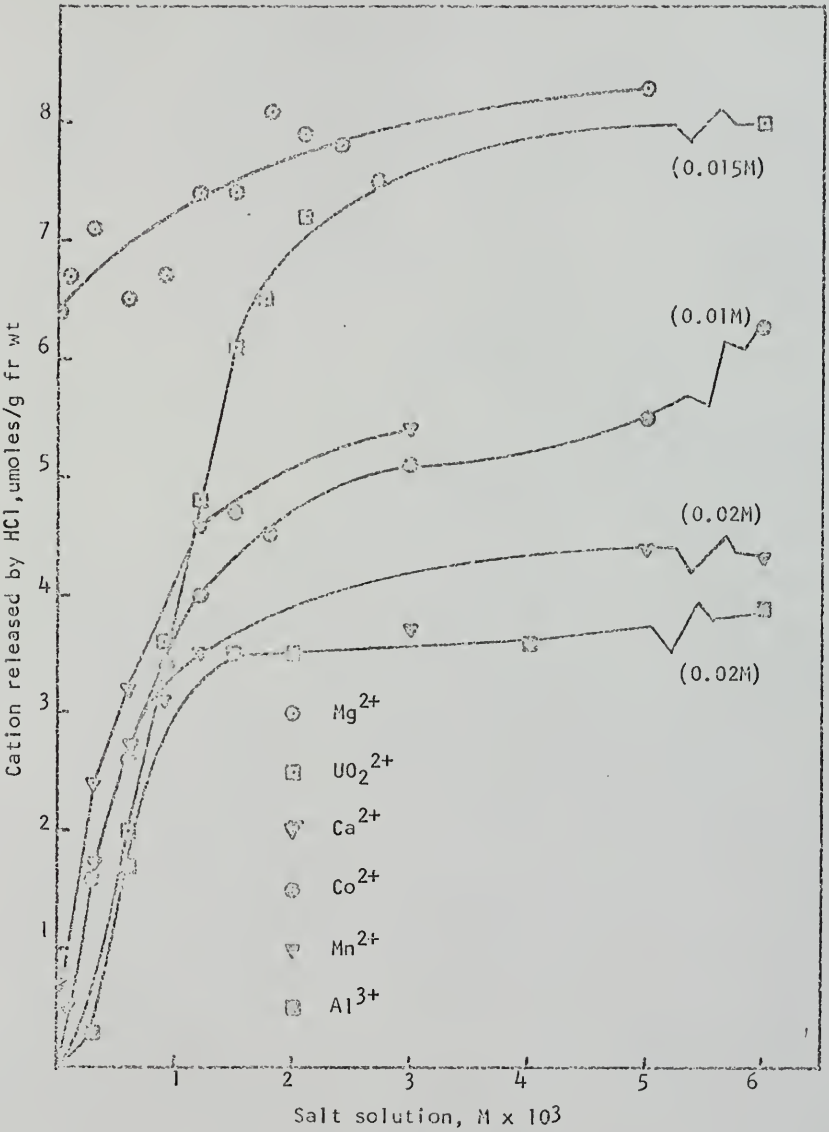


Figure 16. Metal Binding. The schedule for these experiments involved the following: 1 hr in the cation solution (or water control), 3 rinses, 1 hr in water, 3 more rinses, 15 min in 0.01N HCl. The HCl solutions were removed for analysis of the respective cations. Each flask contained 1 g of slices.

points can be made. (a) The ions were bound sufficiently to resist several rinses and a 1-hr incubation in water. (b) A concentration of 0.002M cation solution provided enough ions to saturate the sites in all cases. (c) Appreciable quantities of the ions at low concentrations were bound to the slices. In the case of cobalt (0.0003M), 1.6 umoles of the 3 umoles available were bound by the slices. (d) Magnesium appears to be the ion that normally occupies the sites. The data from Figure 16 show that the slices incubated in water instead of metal cation solutions released 6.5 umoles of Mg^{2+} and 0.75 umoles of Ca^{2+}. (e) Greater quantities of uranyl ion are bound than any of the other ions tested except Mg^{2+}.

In the case of uranyl ion, two experiments were devised to see if the effect on sugar uptake parallels the degree of cation binding to the sites. Figure 17 shows the results of these experiments and it can be seen that maximum inhibition is reached at concentrations of uranyl ion that saturate the binding sites. The inhibition of glucose uptake in this experiment was greater than usual (e.g.,see Figure 9).

If the slices were first treated with HCl and then incubated in metal solutions, the amount of cation binding was considerably reduced. This is shown in Figure 18 for uranyl ion, Mn^{2+}, and Ca^{2+}. Apparently the acid treatment rendered most of the sites unavailable for cation binding. However, the slices took up sucrose as shown in Table 10 with or without replacing the cations. Of course, the possibility exists that the sites involved in sugar uptake were reoccupied by cations from within. The smaller quantity of uranyl ion that binds after acid treatment was sufficient to inhibit uptake as is shown in Table 10.

Work with yeast indicates that metals are displaced from the bind-

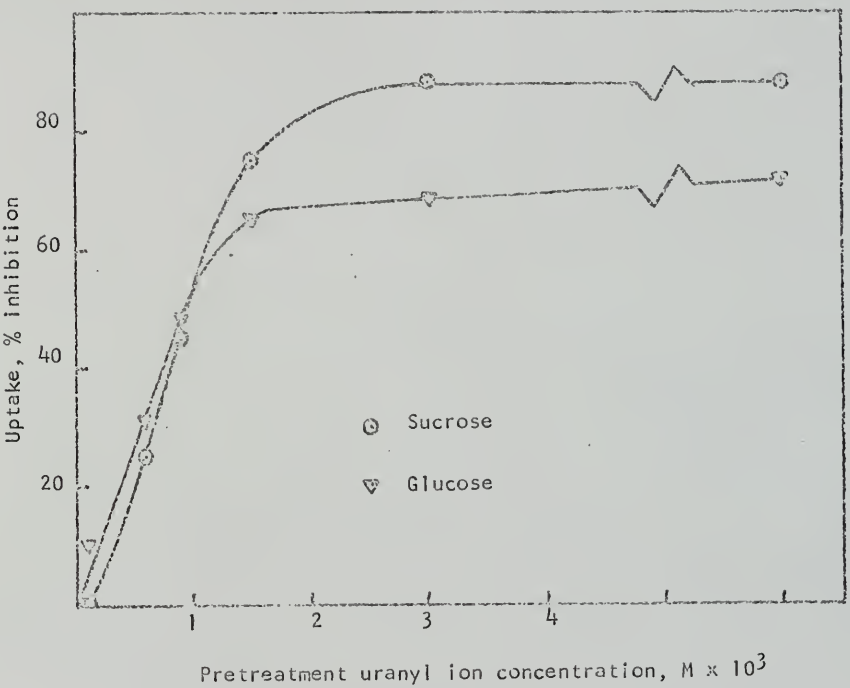


Figure 17. Effect of Uranyl Ion Pretreatment Concentration on Sugar Uptake. In the two experiments reported here the schedules were as follows: 1 hr in the designated concentration of uranyl nitrate, 2 rinses, 30 min in water, 2 more rinses, the uptake solution. The uptake solution was 10 ml of 0.01M sugar in both cases. With sucrose the uptake period was 90 min; with glucose the uptake period was 1 hr. (Whereas the uptake period may affect the degree of inhibition, in this case the experiment was designed to emphasize inhibition as a function of uranyl ion binding and not the amount of inhibition.)

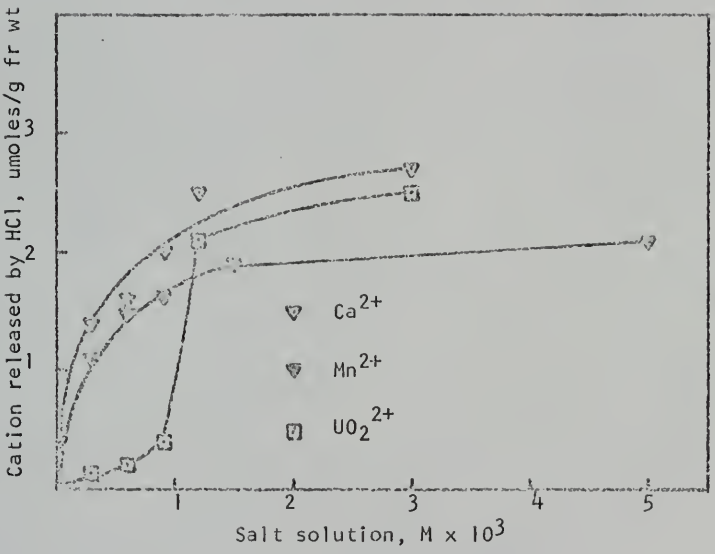


Figure 18. Metal Binding Following an Acid Pretreatment. The slices (1.0 g fr wt) were subjected to the following sequence: 15 min in 0.01N HCl, 1 hr in the metal solution, 2 rinses, water for 1 hr, 2 more rinses, 15 min in 0.01N HCl. The HCl solutions were then removed for analysis of the cations.

ing sites during sugar uptake and are again bound after the sugar has been taken up (53). Several experiments which involved pretreatment with Co^{2+} or Mg^{2+} failed to detect this phenomena with slices of the corn scutellum.

As described in the methods section, corn was normally grown in tap water. In one experiment corn was grown in distilled water. Sucrose uptake by slices from this group of seedlings was about the same as with slices from tap-water-grown seedlings (i.e., 17 umoles/g hr from 0.01M sucrose). Pretreatment with salts of Ca^{2+}, Mg^{2+}, or K^{+} had little effect on sucrose uptake into slices from seedlings grown in distilled water.

DISCUSSION

From the results of this study the following conclusions are drawn. (a) Sucrose is taken up actively without inversion. (b) Hexoses are taken up by two processes operating simultaneously, diffusion and active transport. (c) The active uptake mechanisms for sucrose and the hexoses are located at the plasmalemma. (d) The active uptake mechanisms for both sucrose and the hexoses are driven by glycolysis. (e) The characteristics of metal binding as related to sugar uptake are quite different in the corn scutellum when compared to those of yeast cells.

Several lines of evidence have led Humphreys and Garrard (65) to the conclusion that sucrose is taken up without inversion by the corn scutellum. Results obtained in this work support that conclusion. (a) The amount of extracellular inversion was insufficient to support uptake at the observed rate. (b) The kinetics of hexose uptake are different from the kinetics of sucrose uptake (cf. Figure 4 and Figure 8). (c) The rate of fermentation in sucrose was less than would be expected (Table 3) if inversion occurred either prior to or during uptake. (d) If inversion preceded uptake, the effect of uranyl ion on sucrose should be similar to the effect on hexose which is not the case (cf. Table 4 and Figure 9). (e) The ratio of accumulated sucrose to sugar taken up is higher when sucrose is supplied than when glucose is supplied (p. 52).

The maltose data (Table 7) show that maltose is also taken up by the tissue without hydrolysis.

That sucrose uptake is an active process is indicated by the fact that it is taken up against concentration gradients. Slices that contained 133 umoles sucrose/g (0.13M sucrose) will take up sucrose from 0.01M solution (Table 9). Slices incubated in water for 1 hr contained 68 umoles sucrose/g (0.068M sucrose) and will take up sucrose from 0.001M sucrose (Figure 2). The tissue sucrose concentrations given above are minimum values since an equal distribution of sucrose throughout the tissue water is assumed; compartmentation of sucrose would increase the ratios. The scutellum is composed of mesophyll parenchyma, an epithelial layer and vascular tissue. It is assumed that most uptake occurs in the parenchyma cells since these cells appear to make up about 80-90% of the scutellum.

The inhibition of sucrose uptake by DNP is consistent with the idea of an active, metabolic energy-requiring process.

The inhibition of sucrose uptake by turanose (and probably by lactose) fulfills one of the criteria given for a facilitated diffusion or an active transport process (1).

The data in Table 10 clearly show that a 15-min rinse in 0.01N HCl does not disrupt the normal functioning of the cells insofar as uptake is concerned. Since this treatment removes uranyl ion from the cells and restores the ability of the cells to take up sugar (67), it appears that the effect of uranyl ion is at the cell surface. It is concluded that the mechanism for sucrose uptake is located at the plasmalemma and the same argument can be applied to the active portion of glucose uptake (see below). In contrast to these results, Sacher (14) found that uranyl ion had no effect on sugar uptake. Humphreys (personal communication) has shown that slices are not damaged by high concentrations of fructose. When placed in 2.0M fructose slices will synthesize

sucrose. In contrast, pretreatment of slices in 1.0M sucrose caused the slices to appear flaccid and rendered them incapable of synthesizing sucrose when placed in 0.01M fructose. These observations support the contention that the external membrane is permeable to hexose but not to sucrose.

However, the results of this thesis indicate that the scutellum also has an active hexose transport mechanism. The constant rate of glucose uptake until the bathing solution has been depleted (Fig. 10) cannot be explained by diffusion alone and yet many data have been accumulated to show that the cytoplasm is free space to hexoses. The combination of diffusion and an active transport mechanism can explain these results. The hexose uptake vs concentration curves might also be explained as a combination of diffusion and active transport. The curve (Fig. 10) of glucose uptake (vs time) after uranyl ion treatment is a typical diffusion curve. It is true that this curve could also be the result of a process which follows enzyme kinetics provided that the Km is high in comparison to the concentration. However, the substrate concentration was 0.01M glucose which would require quite a high Km. Since uranyl ion blocks the uptake of sucrose almost completely and partially blocks glucose uptake it appears that uranyl ion is blocking the active portion of glucose uptake. The uptake rate vs concentration curves for the hexoses (Figs. 8 and 9) 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 glucose concentrations diffusion kinetics are represented; i.e., a straight line is obtained.

Glucose is not accumulated by the scutellum cells but is used to

form sucrose or is catabolized. It is assumed that the glucose which diffuses into the tissue is phosphorylated via hexokinase while that glucose taken up actively is phosphorylated at the plasmalemma. This is active transport in the sense that uptake is being energetically driven 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 would be called group translocation by Roseman (57).

A combination of active transport and facilitated diffusion is thought to be involved in the uptake of glucose by yeast (55). Reinhold and Eilam (26), 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 idea that all of sucrose uptake is active whereas half of glucose uptake is active and half passive is supported by the following results. (a) At concentrations of 0.01M sugar and 1×10^{-3}M phloridzin the inhibition of sucrose uptake was twice that of glucose uptake (Table 5). (b) Sucrose uptake was inhibited twice as much by DNP as was glucose uptake. (c) The effect of anerobic conditions (p. 55) was to inhibit sucrose uptake twice as much as glucose uptake; however, the inhibition of sucrose uptake was not complete.

It is concluded that the active uptake mechanisms of glucose and sucrose are driven by glycolysis. That glycolysis can drive uptake is indicated by the degree to which uptake proceeded under N_2 (p. 55). That O_2 availability limits accumulation of sucrose but does not limit uptake (p. 65) is a further indication that glycolysis can drive uptake. The fact that fermentation is detected even when O_2 is not limiting is an indication that fermentation drives uptake regardless of the avail-

ability of O_2. Younis et al. (25) attributed an increased evolution of CO_2 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 0.1M sugar.

Evidence has been presented to show that fermentation drives sugar uptake. No evidence has been obtained to show that a specific glycolytic step is responsible; however, the data are consistent with a system such as the phosphotransferase system in bacteria where PEP is the energy source for uptake. Garrard and Humphreys (65) studying control of glycolysis in the maize scutellum found no differences in the ATP levels in the presence and absence of fructose. The amount of fructose-P, which stimulates phosphofructokinase, doubled in the presence of fructose but this was considered inadequate to account for a four-fold increase in the rate of glycolysis. The use of PEP in the sugar uptake process might trigger fermentation.

The data in Figure 4 which show sucrose uptake as a function of the concentration in the bathing solution closely fit a Michaelis and Menten curve. This type of data is often presented in support of a carrier uptake mechanism that follows enzyme kinetics. However, the curves that show a linear uptake with time in a bath of decreasing concentration (Figs. 1, 2, and 3) are not at all typical of enzyme kinetics. 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.

At a given concentration the constant uptake rate might be explained as a saturation of the uptake mechanism for sucrose but this mechanism is apparently not saturated until concentrations of about

0.4M (64) which does not explain the constant rate at 0.005M (Fig. 2). It appears that whereas the substrate is not the limiting factor (Fig. 2) the substrate concentration has something to do with the rate of uptake (Fig. 4). When the sugar concentration is maintained at a constant level sucrose uptake increases with time whereas glucose uptake does not (cf. Figs. 5 and 11). A possible explanation for 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 the phosphate donor. When sucrose uptake begins, the phosphate donor is limiting but as uptake proceeds fermentation generates 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. 13). The straight line of sucrose uptake in declining concentration may be the result of a decreasing outside concentration of sucrose being countered by an increasing internal rate of glycolysis and thus an increased internal concentration of the phosphate donor which drives sucrose uptake.

With glucose uptake the amount of diffusion of glucose into the synthesis compartment determines the rate of active transport. There is a competition in the cytoplasm for phosphate donors, ATP or PEP, between hexokinase and the active uptake process. As the external concentration of glucose declines, the amount of diffusion declines thus allowing more phosphate donors to be available for the uptake process. Thus as the concentration declines the active process has available a lower external concentration of glucose for uptake but a higher internal concentration of phosphate donor to drive the 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 external glucose is constant, the amount of diffusion is constant and thus 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. When the glucose concentration available to the scutellum is low the system is capable of removing all of the available glucose quickly; 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 driven by glycolysis also fits the role of the scutellum. The ability to drive uptake under limited O_2 supply would be of obvious advantage to a seed under soil conditions. Garrard and Humphreys (65) have measured a RQ of 3 with whole scutella in air indicating that the scutellum itself might impose conditions of limited O_2 availability.

It is obvious that the metal binding characteristics of scutellum slices differ from those of yeast. The binding of uranyl ion to yeast cells seems to be quite specific to the uptake sites (53). With scutellum slices, however, most of the bound uranyl ion is superfluous to the uptake process as shown by the reduced but still effective amount bound after acid treatment (cf. Figs. 16 and 18, and Table 10). This is in agreement with the results of Wheeler and Hanchey (56) who showed that uranyl ion was bound by oat root tissue at many sites other than the membrane surface. If the release of bound cations occurs during sugar uptake by scutellum slices the amount is too small to be detected with the metal assay procedures used.

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BIOGRAPHICAL SKETCH

Joseph Henry Whitesell was born August 11, 1936, at Clearwater, Florida. In June, 1954, he was graduated from Largo High School at Largo, Florida. In June, 1958, he received the degree of Bachelor of Science with a major in Ornamental Horticulture from Auburn University, Auburn, Alabama. From 1958 until 1960 he served in the United States Navy and was stationed aboard the USS Butternut at Long Beach, California. Following his active duty with the Navy, he worked for a year for the County of Los Angeles as a market inspector. In 1961 he returned to Florida where he worked as an Assistant County Agent in Collier County, and during that time he resided in Naples, Florida. In 1966 he enrolled in the Graduate School of the University of Florida. He worked as a graduate assistant in the Department of Botany and as a research assistant at the Pesticide Research Laboratory until December, 1968, when he received the degree of Master of Science with a major in Botany. From December, 1968, until the present time he has pursued his work toward the degree of Doctor of Philosophy.

Joseph Henry Whitesell is married to the former Jeanne Marie Stenson and is the father of three children.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Thomas E. Humphreys, Chairman
Professor of Botany

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David S. Anthony
Associate Professor of Botany

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Richard C. Smith
Assistant Professor of Botany

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Robert H. Biggs
Professor of Fruit Crops

This dissertation was submitted to the Dean of the College of Agriculture and to the Graduate Council, and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

June, 1971

Dean, College of Agriculture

Dean, Graduate School