The Regulation of Sugar Uptake and Accumulation in Bean Pod Tissue

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Summary. The identity, localization and physiological significance of enzymes involved in sugar uptake and accumulation were determined for endocarp tissue of pods of Kentucky Wonder pole beans (Phaseolus vulgaris). An intracellular, alkaline invertase (pH optimum, 8) was assayed in extracted protein, as well as enzymes involved in sucrose synthesis, namely, uridinediphosphate (UDP-glucose pyrophosphorylase and UDP-glucose-fructose transglucosylase). Indirect evidence indicated the presence also of hexokinase, phosphohexoseisomerase and phosphoglucomutase. The data suggested that sucrose synthesis occurred in the cytoplasm, and that both sugar storage and an alkaline invertase occurred in the vacuole. The latter functions to hydrolyze accumulated sucrose.

An outer space invertase (pH optimum, 4.0) was detected, but was variable in occurrence. Although its activity at the cell surface enhanced sucrose uptake, sucrose may be taken up unaltered.

Over a wide range of concentrations of exogenous glucose the sucrose/reducing sugar ratio of accumulated sugars remained unchanged at about 20. Synthesis of sucrose appears to be requisite to initial accumulation from glucose or fructose, as free hexoses do not increase at the apparent saturating concentration for uptake. Sucrose accumulation from exogenous hexose represents a steady-state value, in which sucrose is transported across the tonoplast into the vacuole at a rate equivalent to its rate of synthesis. Evidence indicates that this component of the accumulation process involves active transport of sucrose against a concentration gradient. The ratio of sucrose/reducing sugars in the accumulated sugars immediately after a period of uptake was inversely related to the level of inner space invertase. Within 16 hours after a period of accumulation, practically all of the sugar occurs as glucose and fructose.

The absence of competition among hexoses and sucrose indicated that a common carrier was not involved in their uptake. From a series of studies on the kinetics of uptake of glucose and fructose, including competition studies, the effects of inhibitors, radioactive assay of accumulated sugars and the distribution of label in accumulated sucrose it appeared that rate limitation for glucose or fructose uptake resides in the sequence of reactions leading to sucrose synthesis, rather than in a process mediated by a carrier protein.

The tissues of several higher plants and some animals are similar in that uptake of glucose is about 3 times faster than fructose (1, 8, 9, 18, 21). For plant tissues glucose inhibits fructose uptake considerably, while fructose does not inhibit glucose uptake (8: Sacher, Hatch and Glasziou, unpublished data on sugarcane). There is considerable evidence from the use of metabolic inhibitors and anaerobiosis that energy coupling is essential to sugar uptake (2, 3, 8). Sugar accumulation from solutions of glucose or fructose occurs principally as sucrose (1, 2, 6, 8, 15, 17).

For yeast and sugarcane it has been shown that hydrolysis of sucrose at the cell surface is prerequisite to uptake (5, 19, 21), while in tobacco leaf discs sucrose is taken up unaltered (16). There are reports of considerable invertase in the outer space of other higher plants (4, 9, 13, 17). In the latter, however, it has not been established that hydrolysis of sucrose is essential to uptake.

Bean endocarp tissue differs from the tissues referred to above in that sugar is stored almost entirely in the form of glucose and fructose, rather than sucrose. Yet in this tissue considerable sucrose is synthesized during the onset of senescence, attending changes in membrane permeability which affect cellular compartmentalization (7). This paper reports on the enzymes involved in sugar uptake and accumulation and their distribution in the tissue. Evidence is presented that the rate-limiting step and energy coupling requirements for hexose uptake may be at the sites of hexose phosphorylation and transformations leading to sucrose synthesis.

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Materials and Methods

The outer exocarp was removed from the pods of mature green Kentucky wonder pole beans (Phaseolus vulgaris). The remaining endocarp, a homogenous tissue, was used for assay of enzymes and uptake and accumulation of sugars.

**Reagents.** Sucrose-U-C14 (6.8 mc/mg), glucose-U-C14 (14.4 mc/mg) and fructose-U-C14 (10.0 mc/mg) were obtained from Volk Radiochemical, Burbank, California; uridinephosphoglucone (UDP-glucose), UTP, ATP and glucose-1-P from Calbiochem Corporation, Los Angeles, California; and analytical invertase from Nutritional Biochemical Corporation, Cleveland, Ohio. Fructosyl-U-C14 sucrose was prepared as described previously (21), using a UDP-glucose-fructose transglucosylase preparation from bean endocarp.

**Preparation of Enzymes.** Endocarp tissue was ground with glass beads in a chilled mortar, the homogenate expressed through fine muslin, and the resultant juice centrifuged at 30,000 × g at 2° for 30 minutes. Enzyme prepared from the supernatant fraction by precipitating protein with ammonium sulfate (described in text) was used for determinations of Km's and levels of enzymes.

**Assay of Enzymes.** All enzymes were assayed using radioactive substrates. Aliquots (5 μl) of reaction mixtures were applied at zero time and at various intervals onto Whatman No. 1 paper and chromatographed with nonradioactive sucrose, glucose and fructose markers, using descending chromatography with ethyl acetate: pyridine: water (8:2:1, v/v) as the eluting solvent. Chromatograms were developed with p-anisidine phosphate and radioactive sugars assayed directly on the papers with a thin end-window Geiger-Müller tube. Enzyme activity was measured during the zero order phase of the reactions.

Sucrose synthesis was assayed in 0.2 ml reaction mixtures containing enzyme (0.05–0.2 mg protein), 0.05 ml 0.2 M Tris-HCl, pH 8, additives as indicated below and incubated at 30° in a shaker. Tris (0.05 M) was used as it inhibited (94%) the alkaline invertase in this tissue, as was shown for sugarcane (11). For assay of UDP-glucose-fructose transglucosylase, reaction mixtures contained 1.0 μmole UDPG and 1.3 μmole fructose-U-C14 (1.2 μc). For detection of UDP-glucose pyrophosphorylase reaction mixtures contained 2.5 μmoles UTP, 5 μmoles glucose-1-P, 2 μmoles MgCl₂ and 1.3 μmole fructose-U-C14 (1.2 μc). Sucrose phosphorylase was assayed in reaction mixtures containing 5 μmoles glucose-1-P and 1.3 μmole fructose-U-C14 (1.2 μc). Enzyme activity was measured by counting the baseline spots before chromatography, the sucrose spots after chromatography, and calculating sucrose synthesis as the percentage of baseline radioactivity appearing in sucrose.

Invertase was assayed in 0.2 ml reaction mixtures containing enzyme (0.1–0.7 mg protein), 5.9 μmoles sucrose U-C14 (1.2 μc) and 0.05 ml phosphate-citrate buffer, pH 8.0. Procedures for assay of invertase in cell-residue fractions are described in the text.

For assay of invertase in the outer space, cannula sections (1.2-mm diameter) from endocarp tissue were washed for 1 hour in running tap water. Batches of 200 mg fresh weight were incubated for 3 hours in 0.3 ml reaction mixtures in 10 × 70-mm test tubes at 30° in a shaker. Reaction mixtures, buffered to various pH's, contained 9 μmoles sucrose-U-C14 (1.5 μc). At zero time and various interval aliquots (5 μl) were applied to Whatman No. 1 paper for radioactive assay of sucrose hydrolysis.

**Sugar Uptake and Accumulation.** Uptake describes the total amount of sugar removed from the medium and retained by the tissue after washing for 1 hour, irrespective of its form (21). This was assayed in a 70% ethanol extract. That part of the total uptake which appeared as sugar in the ethanol extract is defined as accumulation. Active transport describes transfer of sugars against a gradient. Tissue sections were prepared and washed as described above. Batches of 200 mg each were incubated in a shaker for 3 hours at 30° in 0.3 ml of radioactive sugar solutions. Unless otherwise specified, uptake was assayed in reaction mixtures buffered at pH 6.1. After incubation the tissue sections were washed for 1 hour in running tap water, blotted gently, and extracted with 3 volumes of 95% ethanol overnight in a shaker. For determination of uptake, aliquots of the ethanol extract were applied onto planchets and counted with a Baird-Atomic thin end-window gas-flow counter. For determining the form in which sugar was stored, aliquots of the ethanol extract were applied to Whatman No. 1 paper and chromatographed as described above, followed by radioactive assay of glucose, fructose and sucrose.

The distribution of radioactivity in the hexose moiety of stored sucrose was assayed as described previously (20).

**Results.**

**Invertase.** An invertase was determined in the supernatant fluid from a homogenate of endocarp tissue after centrifugation at 30,000 × g. The enzyme was characterized using protein precipitated from the supernatant fraction with 35% (w/v) ammonium sulfate. The enzyme is optimally active over a broad pH range from 7.5 to 10 (fig 1), and shows 66% of maximal activity at pH 7. The Km for this alkaline invertase was determined to be 2.6 × 10⁻³ M. The amount of enzyme activity varied seasonally, generally from 1.2 to 5.1 μmoles sucrose hydrolyzed/g fresh weight per hour, although in 2 of 13 assays conducted over a 1-year period no enzyme was detected. The enzyme is similar to an invertase occurring in sugarcane (11) in its Km for sucrose and in being inhibited (94%) by 0.05 M Tris, pH 8.

When endocarp sections were incubated in buffered solutions containing 0.03 μCi sucrose-U-C14, considerable free glucose and fructose sometimes ap-
peared in the ambient solution. The increase in sucrose hydrolysis with decrease in pH was paralleled by increased uptake (fig 2). Similar results were obtained in 2 other experiments. A pH lower than 4 was not used for fear of injury to the tissue. That invert sugars formed a considerable part of the uptake may be indicated also by the similarity shown between the plot for free space invertase activity and the plot for free space invertase activity adjusted for uptake (fig 2). For the latter, radioactivity taken up was added to outer space invertase activity measured by assay of reducing sugars in the ambient solution.

To ascertain the site of the invertase a sample of the tissue was homogenized and the juice expressed through fine muslin. The dialyzed juice contained only the alkaline invertase. The remaining cell-residue fraction was washed 3 times with deionized water and then assayed for sucrose hydrolysis in standard reaction mixtures for invertase assay. Buffered at various pH's (fig 3). The pH-activity curve indicated that the hydrolysis was enzymic. The similarity in specific activity (μmoles sucrose hydrolyzed/g fr wt per hr) of sucrose hydrolysis at pH 4.0 by tissue sections and by washed cell-residue preparations (compare fig 2 and 3) indicated that under both conditions the inversion of sucrose was mediated by the same enzyme and that the enzyme is therefore in the outer space.

Assays for outer space invertase in bean endocarp over a 1-year period showed that among 12 assays the enzyme level was high (0.6–2.5 μmoles sucrose hydrolyzed/g fr wt per hr) in 3 samples, low in 4 and not detectable in 5 batches of tissue. The basis for this variability is not known. The pH optimum of the enzyme detected in the outer space and cell residue fraction was markedly different from that of the intracellular invertase, which indicated that its origin could not be attributed to loss of intracellular enzyme.

Although the occurrence of outer space hydrolysis of sucrose may enhance uptake from a solution of sucrose (fig 2), the tissue is not completely dependent on

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**Fig. 1.** pH-Activity curve for inner space invertase of bean endocarp. Tris-HCl, 0.05 M, pH 8.0, inhibited hydrolysis of sucrose 94%.

**Fig. 2.** pH-Activity curve for outer space sucrose hydrolysis and sucrose uptake. Washed tissue sections (0.2 g) were incubated in 0.3 ml solutions of 0.03 M sucrose-UC14 at various pH's. Δ = sucrose uptake assayed as radioactivity in ethanol extract; X = outer space invertase activity measured by assay of invert sugars in ambient solution; O = relationship shown between hydrolysis of sucrose in the outer space and uptake by plotting the sum of uptake plus outer space invertase activity assayed as described above.

**Fig. 4.** pH-Activity curve for sucrose hydrolysis by washed cell residue fraction of tissue used for assay of outer space invertase activity in figure 2.
hydrolysis prior to uptake as in yeast (5, 19) or sugar cane (21). This is illustrated by the results of several experiments (Table I) which indicate that sucrose may be taken up readily in the absence of outer space invertase activity.

SUCROSE SYNTHESIZING ENZYMES. The 2 terminal enzymes in the pathway of sucrose synthesis, namely UDP-glucose pyrophosphorylase and UDP-glucose-fructose transglucosylase, were demonstrated in bean endocarp, assayed in ammonium sulfate-precipitated protein fractions in the presence of 0.05 m Tris, pH 8.0. The latter enzyme was distributed about equally between 35% and 35 to 70% ammonium sulfate fractions. For both enzymes sucrose synthesis was determined by assay of the incorporation of radioactive fructose-U-C\(^{14}\) into sucrose. For synthesis of sucrose involving UDP-glucose pyrophosphorylase, ATP could not substitute for UTP. Hydrolysis of the sucrose synthesized and subsequent assay of the radioactive invert sugars showed all of the radioactivity in the fructose moiety. The activity of UDP-glucose-fructose transglucosylase varied from 0.62 to 4.7 (av = 1.1) μmoles sucrose synthesized/g fresh weight per hour. Activity of UDP-glucose pyrophosphorylase (assayed in a 70% ammonium sulfate fraction) was measured by the rate of sucrose formation from glucose-1-P, UTP, fructose-U-C\(^{14}\) and MgCl\(_2\). The formation of 0.34 μmole sucrose/g fresh weight per hour indicated that UDP-glucose fructose transglucosylase was nonlimiting, as the activity of the latter enzyme is substantially higher than this. Synthesis of sucrose from fructose-U-C\(^{14}\), UTP and MgCl\(_2\) in a 70% ammonium sulfate fraction occurred at a low rate (0.14 μmole/g fr wt per hr), which could have been due in part to high phosphatase activity, inasmuch as considerable free glucose was formed. The synthesis of sucrose under these conditions indicates the presence in the tissue of hexokinase, phosphohexose isomerase and phosphoglucomutase. The rate at which sucrose is accumulated by tissue from a solution of fructose or glucose, as will be shown in a following section, indicates a much greater capacity for sucrose synthesis than has been demonstrated in these cell-free preparations.

No sucrose phosphorylase could be detected in bean endocarp. This enzyme seems to be lacking in higher plants (cf. 11).

### Table I. Relationship between Outer Space Invertase Activity and Sucrose Uptake for Endocarp Tissue Sections

<table>
<thead>
<tr>
<th>Expt</th>
<th>Incubation medium</th>
<th>Outer space sucrose hydrolysis</th>
<th>Uptake sucrose-U-C(^{14})</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>0.03 m sucrose-U-C(^{14})</td>
<td>2.19 g fr wt per hr</td>
<td>0.37 g fr wt per hr</td>
</tr>
<tr>
<td>II</td>
<td>&quot; &quot; &quot; &quot; &quot;</td>
<td>0.99 g fr wt per hr</td>
<td>1.89 g fr wt per hr</td>
</tr>
<tr>
<td>III</td>
<td>&quot; &quot; &quot; &quot; &quot;</td>
<td>0.22 g fr wt per hr</td>
<td>0.90 g fr wt per hr</td>
</tr>
<tr>
<td>IV</td>
<td>&quot; &quot; &quot; &quot; &quot;</td>
<td>0.13 g fr wt per hr</td>
<td>1.31 g fr wt per hr</td>
</tr>
<tr>
<td>V</td>
<td>&quot; &quot; &quot; &quot; &quot;</td>
<td>0.0 g fr wt per hr</td>
<td>0.92 g fr wt per hr</td>
</tr>
</tbody>
</table>

### Uptake and Accumulation from Glucose and Fructose. Sugar taken up by bean endocarp from solutions of glucose-U-C\(^{14}\) or fructose-U-C\(^{14}\) was accumulated as uniformly labeled sucrose (cf. 21). Since bean endocarp contains considerable glucose and fructose it is apparent that sucrose synthesis occurs at a site where the labeled sugar is not significantly diluted by the large pool of endogenous hexoses. Cellular compartmentation of sugars has been reported previously (2, 6, 8, 14, 21). For uptake of either hexose, over a wide range of concentrations (0.005-0.18 m) the sucrose/reducing sugar ratio of accumulated (labeled) sugar scarcely varied from about 20 (Table II). Thus, it appears that sucrose synthesis is a requisite for accumulation from solutions of glucose or fructose, and that the radioactive reducing sugars arise from hydrolysis of sucrose.

### Table II. Relationship between Exogenous Concentration of Glucose, the Rate of Uptake, and the Sucrose/Reducing Sugar Ratio of Sugars Accumulated by Bean Endocarp Tissue Sections

<table>
<thead>
<tr>
<th>Conc of glucose (m)</th>
<th>Glucose uptake (μmoles/g fr wt per hr)</th>
<th>Ratio of C(^{14}) in sugars in sucrose/reducing sugars</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.005</td>
<td>0.43</td>
<td>19.0</td>
</tr>
<tr>
<td>0.01</td>
<td>0.84</td>
<td>19.0</td>
</tr>
<tr>
<td>0.03</td>
<td>1.85</td>
<td>21.2</td>
</tr>
<tr>
<td>0.06</td>
<td>3.38</td>
<td>20.2</td>
</tr>
<tr>
<td>0.12</td>
<td>5.32</td>
<td>24.5</td>
</tr>
<tr>
<td>0.18</td>
<td>5.54</td>
<td>20.0</td>
</tr>
</tbody>
</table>

Glucose uptake was on the average (11 assays) 3 times as fast as that of fructose when comparing uptake from 0.03 m solutions. The Km for fructose uptake averaged 2.1 × 10\(^{-2}\) m for 5 assays (Table III). For uptake of glucose the average Km for 6 assays was 2.1 × 10\(^{-2}\) m, and the range of variability was greater than for fructose. KCN or dinitrophenol at 5 × 10\(^{-4}\) m inhibited glucose uptake substantially (52-84%, table IV). There was no significant difference between the effect of these inhibitors. For potato slices, exposed to similar concentrations of glucose and these inhibitors, total absorption (cpm removed from ambient solution) was inhibited 45% by KCN and 32% by dinitrophenol (14).

### Table III. Km Values for Uptake of Glucose and Fructose by Bean Endocarp Tissue Sections

<table>
<thead>
<tr>
<th>Date of assay</th>
<th>Glucose Km (m)</th>
<th>Date of assay</th>
<th>Fructose Km (m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3/2/64</td>
<td>8 × 10(^{-2})</td>
<td>3/2/64</td>
<td>2.6 × 10(^{-2})</td>
</tr>
<tr>
<td>4/30/64</td>
<td>8 × 10(^{-2})</td>
<td>3/25/64</td>
<td>1.7 × 10(^{-2})</td>
</tr>
<tr>
<td>5/1/64</td>
<td>2.9 × 10(^{-3})</td>
<td>3/31/64</td>
<td>2.2 × 10(^{-2})</td>
</tr>
<tr>
<td>5/7/64</td>
<td>3.3 × 10(^{-3})</td>
<td>4/20/64</td>
<td>1.7 × 10(^{-2})</td>
</tr>
<tr>
<td>5/15/64</td>
<td>3.3 × 10(^{-3})</td>
<td>4/21/64</td>
<td>2.6 × 10(^{-2})</td>
</tr>
<tr>
<td>10/9/64</td>
<td>9.4 × 10(^{-3})</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Determined by Lineweaver-Burk plots.
Table IV. Effect of KCN and 2,4-Dinitrophenol on Uptake of Glucose

<table>
<thead>
<tr>
<th>Expt</th>
<th>Exogenous glucose conc</th>
<th>4 × 10⁻² M KCN**</th>
<th>4 × 10⁻⁵ M Dinitrophenol</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>8.7 × 10⁻² M</td>
<td>81%</td>
<td>84%</td>
</tr>
<tr>
<td>II</td>
<td>1.74 × 10⁻⁴ M</td>
<td>67%</td>
<td>63%</td>
</tr>
<tr>
<td>III</td>
<td>1.0 × 10⁻³ M</td>
<td>52%</td>
<td>53%</td>
</tr>
<tr>
<td></td>
<td>3.0 × 10⁻⁴ M</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Assayed in 70% ethanol extract of tissue after a 1-hour washing.
** Incubated in tightly stoppered 15 ml tubes to prevent loss of KCN.

For yeast cells 10⁻⁴ M uranyl nitrate greatly inhibited uptake and fermentation of sucrose and hexoses, yet respiration of acetate, pyruvate, lactate and ethanol, as well as endogenous respiration was insensitive to uranium (19). From this and other evidence it was concluded that uranium acted at the surface of yeast cells. Since there was no effect of 10⁻⁴ to 10⁻⁸ M uranyl nitrate on uptake of 0.03 M glucose (or sucrose) by bean endocarp, it appears that a different mechanism is involved in sugar permeation of the plasma membrane of yeast cells and cells of higher plants.

Seven experiments were conducted in which tissue sections were incubated in solutions of 0.03 M with respect to both glucose and fructose, with only one of the sugars labeled. Unlabeled fructose had no effect on uptake of glucose-U-C¹⁴, while unlabeled glucose inhibited uptake of fructose-U-C¹⁴ an average of 45%. Lineweaver-Burke plots showed that the inhibition by glucose was competitive (fig 4). In 4 experiments the hexoses galactose or mannose inhibited fructose uptake from equimolar (0.01 M) solutions from 14 to 33%.

A few experiments, however, were contrary to the typical noninhibition of glucose-U-C¹⁴ uptake by unlabeled fructose and the inhibition of fructose-U-C¹⁴ uptake by unlabeled glucose. In one, fructose clearly inhibited glucose competitively (fig 5). The inhibition at 0.03 M concentration of each sugar was 32%, and the Km for glucose uptake was 3.3 × 10⁻² M, which is one order of magnitude below the highest Km reported above (table III) for 6 other determinations. In another instance marked changes were observed in the effect of glucose, galactose and mannose on fructose uptake after short-term storage of whole beans at 5°C. Tissue harvested from a fresh batch of beans showed an uptake of 40 µg fructose/g fresh weight per hour from 0.01 M solution of fructose-U-C¹⁴, which was inhibited 34% by an equimolar concentration of unlabeled glucose. In tissue removed from the beans 2 days later the rate of fructose-U-C¹⁴ uptake had increased 75% and glucose, galactose and mannitol each slightly (ca. 15%) stimulated fructose-U-C¹⁴ uptake. Thus, variations of this kind may result from physiological aging during storage. Such variability and lack of specificity

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Fig. 4. Lineweaver-Burke plot illustrating competitive inhibition of uptake of fructose by glucose in bean endocarp sections.

Fig. 5. Lineweaver-Burke plot depicting atypical competitive inhibition of uptake of glucose by fructose. The Km for glucose uptake was 3.3 × 10⁻² M in this experiment.

appears to speak against a reaction mediated by a carrier protein being rate-determining for uptake of glucose and fructose, and may indicate that the uptake of hexose is metabolically controlled at the site of sucrose synthesis.

In both carrot root and corn root tissue L-arabinose and D-ribose are accumulated as such (8). In bean endocarp L-arabinose and D-ribose (table V) do not inhibit fructose uptake over a wide range of concentrations. It is interesting to note that these pentoses, which do not to a significant extent enter the site of hexose transformations in other plants, offer no competition to uptake of fructose.

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Sucrose Uptake. For studies of sucrose uptake incubation media contained 0.05 M Tris, pH 8.0, which inhibits outer space invertase activity about 90% (a similar molarity of phosphate-citrate buffer inhibited 79%). The Km for sucrose uptake was $3.6 \times 10^{-7}$ M. Apparent saturation occurred at about 0.12 M sucrose. In tissue which had little or no outer space invertase activity there was no inhibition of uptake of labeled sucrose by glucose or fructose, nor was there inhibition in the reciprocal experiments. Thus, it is evident that a common carrier is not involved in uptake of hexose and sucrose. This is in contrast to a report of competitive inhibition of C14-sucrose uptake by glucose as well as for the reciprocal experiments in sugarcane (2), which has since been explained on the basis of sucrose being hydrolyzed during the process of uptake (21).

Although sucrose uptake was enhanced when significant amounts of outer space invertase were present (fig 2), its uptake in the absence of detectable invertase in the free space was often substantial (table I). Thus, it appeared that sucrose could be taken up as such, without need for prior inversion as with sugarcane (21). This is also indicated by the fact that when tissue sections were incubated in fructosyl-U-C14 sucrose (G/F radioactivity ratio = 0.001) the sucrose was taken up and stored essentially unaltered (G/F = 0.03). Further, when tissue sections were incubated in a solution of sucrose-U-C14 along with either nonradioactive glucose or fructose the sucrose in the ethanold extract was symmetrically labeled. Substantial randomization of label occurred in sucrose accumulated under such conditions by sugarcane tissue sections, in which sucrose is hydrolyzed prior to its uptake (21). These results suggest that in bean tissue sucrose may be taken up and accumulated without inversion.

From the following observations, however, it may be demonstrated that some sucrose is hydrolyzed intracellularly during its uptake, the amount depending upon metabolic demands. The ethanol extracts of tissue incubated in uniformly labeled sucrose, glucose or fructose, as well as mixtures of sucrose with an unlabeled hexose, were chromatographed and radioactivity in sucrose and at the origin was assayed on the chromatograms. The radioactivity at the origin was largely sugar phosphates, which do not migrate in the solvent used, and hereafter will be called origin compounds (cf. 9). As shown in table VI the ratio of radioactivity in sucrose compared with origin compounds was 3 to 4 times higher in extracts from tissue incubated in sucrose than in hexose. Further, with a mixture of labeled sucrose and unlabeled hexose in the medium the ratio was increased substantially. The results suggest that when unlabeled hexose is available to meet metabolic demands less sucrose is hydrolyzed intracellularly during its uptake. Although it is not known whether cytoplasmic hydrolysis of sucrose is mediated by invertase or reversal of a UDP-glucose-fructose transglucosylase system, the evidence presented herein on localization of enzymes suggests the latter.

Table VI. Relationship between External Sugar(s) and the Ratio of Radioactivity in Sucrose/Origin Compounds on Chromatograms of Ethanol Extracts of Tissue Sections

| Sugar                  | Unlabeled sugar | Ethanol extract Ratio of cpm in sucrose
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Expt I</td>
</tr>
<tr>
<td>Sucoose-U-C14</td>
<td>Glucose</td>
<td>6.0</td>
</tr>
<tr>
<td>&quot; &quot;</td>
<td>Fructose</td>
<td>15.0</td>
</tr>
<tr>
<td>Glucose-U-C14</td>
<td></td>
<td>10.0</td>
</tr>
<tr>
<td>Fructose-U-C14</td>
<td></td>
<td>2.1</td>
</tr>
<tr>
<td>&quot; &quot;</td>
<td></td>
<td>2.0</td>
</tr>
</tbody>
</table>

Concentrations of $5 \times 10^{-4}$ M KCN and dinitrophenol inhibited uptake from $3 \times 10^{-4}$ M sucrose 81% and 96%, and from $3 \times 10^{-2}$ M sucrose 72% and 88% respectively. Thus, it appeared that these compounds inhibit markedly, irrespective of whether uptake is with or against a concentration gradient.

Significance of Enzymes and their Distribution to Sugar Uptake and Accumulation. Although sucrose uptake is enhanced when free space invertase is present, sucrose may be taken up and accumulated without inversion. Since the tissue contains an intracellular...
ular invertase the observations suggested that this enzyme occurs in the vacuole. Two lines of experimental evidence are consistent with this interpretation. As shown previously, accumulation by tissue sections from solutions of glucose or fructose occurs as sucrose. When such tissue sections were subsequently washed and incubated in a humidity chamber for 16 hours the sucrose/reducing sugar ratio dropped from 22 to 0.6. This observation is consistent with the analyses of fresh tissue, which invariably show that sugar is stored as glucose and fructose, with only traces (sucrose/reducing sugar ratio = 0.037) of sucrose present (7, 23). Since the tissue has a high capacity for sucrose synthesis, which is likely to occur in the cytoplasm because of the need for energy coupling, the vacuolar compartmentalization of invertase and sugar storage is indicated (cf. 6, 21). Also, several experiments in which both the sucrose/reducing sugar ratios and the level of intracellular (alkaline) invertase were assayed showed an inverse relationship between the amount of this enzyme and the sucrose/reducing sugar ratio of the accumulated sucrose (table VII). When the level of intracellular invertase was high (3.0-5.1 μmoles sucrose hydrolyzed/g fr wt per hr) the ratio was about 2.0, while with a low level of invertase (0.6-1.0 μmole) the ratio was about 26. This refers to the ratios immediately after removal of the tissue from the sugar solution. As noted above, the sucrose/reducing sugar ratio decreases greatly when the tissue sections are incubated subsequently in a humidity chamber for 16 hours. Similar observations were made for sugar accumulation in sugarcane (21: Sacher. Hatch and Glaziov, unpublished data).

Discussion

Four lines of evidence indicate that sucrose may be taken up and accumulated without hydrolysis. First, fructosyl-labeled sucrose was taken up and stored without significant alteration of its labeling (see also 16). Secondly, sucrose accumulated from solutions of mixtures of sucrose with either glucose or fructose was uniformly labeled, irrespective of which of the 2 sugars in the external solution was labeled. Were sucrose hydrolyzed during accumulation some randomization of label could be expected at the site of phosphorylation and conversion of hexoses leading to sucrose synthesis (cf. 21).

Thirdly, it was demonstrated (20) that the incubation of bean endocarp tissue sections in fructose-U-C14 and UDP-glucose, conditions favorable for synthesis of fructosyl-labeled sucrose extracellularly, caused a marked decline from unity of the G/F radioactivity ratio of the accumulated sucrose, which was proportional to the amount of sucrose synthesized extracellularly. The results were explicable in terms of uptake and accumulation of fructosyl-labeled sucrose unaltered, and of fructose-U-C14, of which the latter was converted intracellularly to uniformly labeled sucrose.

Fourthly, the ratio of label in sucrose compared with origin compounds was about 3-fold greater in ethanol extracts of tissue incubated in sucrose than in glucose or fructose (table VI). This ratio was increased substantially when either unlabeled glucose or fructose was in the incubation medium with sucrose-U-C14. These observations indicate that intracellular hydrolysis of sucrose during uptake is controlled by the relative demand for hexose. It appears that the presence of hexose increases the ratio of sucrose/origin compounds by providing a readily source of metabolic intermediates. For tobacco leaf discs part of the evidence used in concluding that sucrose was taken up without hydrolysis was the much lesser starch formation from exogenous sucrose than from glucose (16).

Several points may be enumerated which provide evidence about cellular localization of sugar storage, inner space invertase and sucrose synthesizing enzymes.

1) In fresh cut tissue the endogenous sugars consist largely of glucose and fructose, and only trace amounts of sucrose (7, 23). Since the sucrose accumulated by bean endocarp from solutions of uniformly labeled glucose or fructose is symmetrically labeled, it appears that the endogenous hexoses do not dilute the metabolic pool where sucrose synthesis occurs.

2) It could be shown from assays of tissue extracted with hot ethanol immediately after removal from a solution of radioactive sugar and washing, that the sucrose/reducing sugar ratio of the accumulated sugars is inversely related to the level of inner space invertase (table VII). Further, practically all of the sucrose accumulated is hydrolyzed within 16 hours, after the period of accumulation, as shown by the marked decrease in the sucrose/reducing sugar.
ratio (from 22-0.6), when tissue sections are stored overnight in a humidity chamber after uptake from a solution of labeled hexose or sucrose.

3) In senescing bean endocarp tissue considerable sucrose is synthesized associated with changes in permeability and leakage of sugars (7). This observation indicated that the hexoses are normally separated from the site of sucrose synthesis by a permeability barrier, probably the vacuolar membrane. It may be indicated from the evidence that sucrose is synthesized when hexoses stored in the vacuole leak into the cytoplasm.

It is concluded that both sugar storage and the inner space invertase occur in the same compartment, which is believed to be the vacuole (cf. 6, 16, 21). Considering the high capacity of endocarp tissue for sucrose synthesis, it is difficult to envisage the hydrolysis of accumulated sucrose and storage of reducing sugars occurring in the compartment where synthesis of sucrose takes place. The energy coupling and number of enzymes involved in sucrose synthesis bespeaks its occurrence in the cytoplasm.

Differences in the rates of uptake and competition among glucose, fructose and sucrose have led to suggestions that the rate-limiting reaction for sugar uptake involves a common carrier which has a greater affinity for one sugar than another (2, 8). Data from the present study, however, suggest that the rate-limiting reaction(s) reside at the site of hexose phosphorylation and transformations leading to sucrose synthesis (cf. 6). Following are summarized a number of lines of evidence which might be used for an evaluation of these 2 alternative interpretations.

1) Glucose is taken approximately 3 times faster than fructose. 2) Glucose inhibits fructose-U-C^14 uptake, while fructose normally has no effect on glucose uptake. 3) L-Arabinose and D-ribose (table V), which are accumulated as such (8), and thus do not enter the pool of hexose transformations, offer no competition to uptake of fructose. 4) Cyanide and dinitrophenol greatly inhibit uptake of hexose. The foregoing observations are consistent with either a carrier-mediated process or an enzymic process at the site of sucrose synthesis being rate-limiting to hexose uptake. The following observation is consistent with the interpretation that rate limitation occurs at the site of reactions leading to synthesis of sucrose.

Over a wide range of concentrations of exogenous glucose (0.005-0.18 M) the sucrose/reducing sugar ratio of the accumulated sugars remains essentially unchanged at about 20 (table II). If the rate-limiting reaction for glucose or fructose uptake was carrier-mediated, it might be expected that at the apparent saturating concentration for hexose uptake (table II) considerable free glucose or fructose would occur among the accumulated sugars. The results indicate that sucrose synthesis is a requisite for accumulation from a solution of glucose or fructose. Similar results were observed for sugarcane (6).

The observations listed next are inconsistent with competition at a carrier-site being an explanation for the faster uptake of glucose than fructose and the inhibition of fructose uptake by glucose. 1) The lower Km for fructose uptake (2.1 x 10^-2 M) than glucose (2.0 x 10^-1 M) would suggest fructose as a good inhibitor of glucose uptake, when the reverse is usually observed. 2) The variations in the Km for glucose uptake of one order of magnitude appear unusually high for variations in the Km of a reaction mediated by a carrier protein. Such variation might be expected if the apparent Km's are actually measurements of the rate of sucrose synthesis from glucose, and the amount of a rate-limiting enzyme among the enzymes involved (e.g., affecting glucose phosphorylation) is subject to variation. 3) Of 7 determinations of the Michaelis constant for glucose uptake, only with the lowest observed Km (apparent) was the inhibition of glucose by fructose observed (fig 5). It does not seem that the specificity of a carrier protein common to both glucose and fructose would change to this extent, as glucose normally inhibits fructose uptake substantially. The observation, however, would seem consistent with what is suggested above.

Most of the observations above are explicable in terms of glucose normally being a better source of glucose-1-phosphate and fructose than fructose is of glucose-1-phosphate. It is suggested that the variable kinetics observed for uptake of glucose and fructose and mixtures of hexoses may be due to variations in the amounts of enzymes in the sequence for sucrose synthesis shown below, and thus to differential effects of enzyme products on reactions in the sequence. This could result in one or another of the reactions being rate-limiting. One possibility is that glucose or glucose-P inhibits fructose phosphorylation.

\[
\begin{align*}
G & \xrightleftharpoons{F-6-P \rightarrow G-6-P \rightarrow G-1-P} \\
G-1-P + UTP & \rightarrow UDPG + P_i \\
UDPG + F & \rightarrow Sucrose
\end{align*}
\]

Immature sugarcane tissue resembles bean pod tissue in the relative rates of glucose and fructose uptake and inhibition of fructose uptake by glucose. When sugarcane discs were provided labeled fructose and unlabeled glucose the G/F radioactivity ratio of the sucrose accumulated was 0.67, while the ratio ranged from 1.58 to 3.65 for the reciprocal experiments. Also marked asymmetry occurred in sucrose accumulated from mixtures of labeled sucrose and unlabeled hexoses. In this tissue sucrose is hydrolyzed in the free space prior to being taken up. When the hexose was fructose the G/F ratio was 3.25, while with glucose as the unlabeled sugar the ratio was 0.98 (21; Sacher, Hatch and Glassiou, unpublished data). For this tissue the observed asymmetry of accumulated sucrose also appeared to be explicable in terms of glucose being a better source of glucose-1-P and fructose than fructose is of glucose-1-P.

In animal tissue, where glucose uptake is also faster than that of fructose, the phosphorylation-rate ratios for glucose and fructose in vivo are very sim-
ilar to their absorption-rate ratios in vivo (12), and glucose and glucose-P strongly inhibit fructose phosphorylation, while fructose inhibition of glucose phosphorylation is hardly detectable (22). The phosphorylative capacity of bean endocarp for glucose and fructose in vitro has not been investigated yet.

The experimental work thus far with bean tissue does not unambiguously negate the possibility of a common carrier being rate-limiting to uptake of glucose and fructose. Nonetheless, several lines of evidence presented, which appear to be reflections of the kinetics of reactions in the cytoplasm, are consistent with the hypothesis that the rate-limiting reaction to hoxose uptake resides at the site of sucrose synthesis. If this is so, the possibility is presented that the cytoplasm is free space to hoxose over the range of concentration used (0.005-0.18 M). This has been suggested previously for sugarcane (6), without implying that the cytoplasm is free space to other solutes.

As noted previously the sucrose/reducing sugar ratio in ethanol extracts made immediately after the 3-hour period of sucrose or hoxose uptake (table VII) is inversely related to the level of inner space (vacuolar) invertase. From this and other evidence presented on cellular compartmentation it may be deduced that sucrose accumulation from exogenous glucose represents a steady-state value, in which sucrose synthesized from glucose is transported across the tonoplast into the vacuole at a rate equivalent to its rate of synthesis in the cytoplasm. The initial concentration of sucrose in the tissue (7) is about 3.4 × 10⁻³ M (or 1.2 mg/g fr wt). The rate of accumulation of sucrose from exogenous glucose ranges from 0.07 to 0.9 mg/g fresh weight per hour (table II), of which only a fraction would be present in the cytoplasm at any time. Thus, it appears that the steady-state accumulation involves active transport of sucrose across the tonoplast. The mechanism by which sucrose is transported across the tonoplast against a concentration gradient in bean endocarp is not known. For sugarcane it appears that the energy for such a process is derived from hydrolysis of sucrose-P synthesized from hoxose by UDP-glucose-fructose 6-phosphate glucosyltransferase (10).

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Literature Cited