Direct Evidence for Translocation of Sucrose in Sugarcane Leaves and Stems

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Introduction

Quantitative analysis shows that sucrose is the major organic constituent of phloem exudates in most plants (6, 8). When leaves are allowed to photosynthesize in C14O2 for short periods most of the translocated radioactivity is generally found in sucrose (6, 8). With respect to the nature of the translocated compound in these plants there are 3 possible hypotheses which are consistent with the above observations. Either A) sucrose is the translocated compound, B) the substance translocated is a derivative of sucrose which is synthesized without breakdown of the sucrose molecule, and which is in rapid equilibrium with sucrose in the phloem such that only small quantities of the sucrose derivative are present, or C) a molecule derivable from sucrose only through breakdown is the transit molecule but again the equilibrium relationship with sucrose being such that only a small quantity is present. The possibility that transit molecules in the phloem may be present in small quantities compared with nontransit molecules has been emphasized by Swanson (6) and others (3).

The results reported herein exclude the operation of hypothesis C) for translocation in sugar cane.

Methods and Materials

A hybrid sugarcane variety (Pindar') was used. Plants were grown for approximately 12 weeks in a greenhouse then 5 days prior to the experiments were moved to another greenhouse maintained at 29°. Fructose-U-C14, glucose-U-C14 and sucrose-U-C14 were obtained from the Radiochemical Centre, Amersham. Yeast invertase was purchased from Difco Laboratories. Fructosyl-U-C14 sucrose was prepared as previously described (5).

Translocation Studies. Radioactive compounds were applied to the leaf subtended by the top visible dewlap, application commencing at about 9 AM. Plants were in full sunlight at 29°.

For the application of C14O2 the apical 20 cm of an 80 cm leaf was enclosed in a plastic bag containing 4 mg of BaC14O2 (0.4 mc) in a steel planchet. C14O2 was released by the addition of 20% acetic acid with a hypodermic syringe.

Solutions of radioactive sugars were applied to leaves 60 cm from the dewlap in 1 of 2 ways. In some experiments solutions were applied to a 1 cm length of the upper surface of the midrib after 4 longitudinal cuts to a depth of approximately 0.2 cm had been made in the ventral epidermis to facilitate entry of the solutions. This treatment did not damage the vascular tissue which is situated adjacent to the dorsal epidermis. Midrib treated in this way will be referred to as nonsevered midrib. Alternatively the leaf blade was cut 60 cm above the dewlap and the sugar solutions applied to the cut end of the midrib.

At the conclusion of the translocation period, the 1 cm section of the leaf below the point of application was discarded then consecutive 5 cm sections of the midrib or sheath were placed in 3 volumes of 95% ethanol, heated at 100° for 10 minutes and extracted by shaking for 48 hours.

Analytical Procedures. To determine total radioactivity, aliquots of the ethanol extracts were plated on glass planchets and counted in a gas-flow counter. Further samples of the ethanol extract were chromatographed on paper using ethyl acetate: pyridine: water (8:2:1) as a developing solvent. After locating their position with p-anisidine phosphate, the radioactivity in sucrose, glucose, and fructose was determined as previously described (2). With this solvent hexose phosphates, malate, citrate, aspartate and glutamate are among the compounds which remain at the origin. Radioactivity which chromatographed in the area corresponding to sucrose was isolated and treated with yeast invertase. Following invertase treatment all the radioactivity chromatographed with glucose and fructose. By this procedure the identity of sucrose was confirmed and the ratio of C14 in the hexose moieties of sucrose determined. The method for determining the sucrose and reducing sugar content of tissues was described previously (1).

Results and Discussion

Translocation of Photosynthate. After the upper part of a leaf was exposed to C14O2 for 15 minutes, the radioactivity in the lamina and midrib below the application zone was determined (fig 1). The radioactivity in the lamina decreased logarithmically with distance from the application zone but in the midrib most radioactivity was located 15 to 20 cm away. The distribution in the midrib is consistent with label entering the midrib from the veins of the lamina, which in sugarcane converge to the midrib.

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After 15 minutes approximately 93% of the ethanol-soluble radioactivity of the lamina and midrib in the application zone was located in sucrose. The remainder did not move from the origin of chromatograms with the solvent used (see Methods and Materials section). Of the radioactivity translocated to the lamina and midrib below the application zone approximately 99% was in sucrose.

Translocation of Radioactive Sugars. First, information was obtained on the kinetics of movement and composition of translocated radioactivity when labeled sugars were applied to the leaf midrib. The distribution of label in the midrib below the point of application was similar to that for the lamina shown in figure 1, irrespective of the radioactive sugar supplied or the method of application. Compared with application to the nonsevered midrib, about 4 times as much radioactivity was translocated when sugar solutions were supplied to the cut end of the midrib. In 5 experiments the amount of material translocated from solutions of sucrose was between 30% and 50% of that from equimolar solutions of glucose or fructose. Movement of glucosamine-U-C$^{14}$ was barely detectable. With solutions of sucrose or hexoses containing approximately $7 \times 10^9$ dpm/0.1 ml the translocation velocity was between 4 and 6 cm/minute. A velocity of 4.5 cm/minute was observed for movement of photosynthate.

The results of experiment 1 in table I show the relative distribution of translocated radioactivity in different compounds when radioactive sucrose or hexoses were supplied to the nonsevered midrib. Essentially all the translocated radioactivity was located in sucrose if sucrose was the applied sugar. When labeled glucose or fructose were supplied at a concentration of $7 \times 10^{-3}$ M a large part of the translocated radioactivity was isolated in the applied hexose, lesser amounts occurring in sucrose and compounds which remained at the origin of chromatograms. However, when glucose-U-C$^{14}$ was supplied at a concentration of $0.7 \times 10^{-3}$ M most of the radioactivity appeared in sucrose.

To establish whether labeled hexoses were converted to sucrose during translocation, or prior to entry into the conducting tissue, fructose-U-C$^{14}$ was supplied to 4 leaves over a period of 10 minutes then the composition of translocated radioactivity ex-

![FIG. 1. Distribution of radioactivity in the lamina and midrib below a zone of the leaf exposed to C$^{14}$O$_2$ in the light for 15 minutes. Experimental details are described in the Methods and Materials section.](image)

### Table I

**Distribution of Translocated Radioactivity in the Midrib of Leaves Supplied with Labeled Sugars**

An excess of the sugar solutions was supplied to the nonsevered midrib and translocation was allowed to proceed for 15 minutes. The solutions contained approximately $7 \times 10^9$ dpm/0.1 ml. Analyses were for sections of the midrib between 5 and 10 cm below the application area. Other experimental details are described in the Methods and Materials section.

<table>
<thead>
<tr>
<th>Expt.</th>
<th>Radioactive sugar supplied</th>
<th>Conc. (M)</th>
<th>Total C$^{14}$ in the midrib section* (cpm)</th>
<th>Percent of total radioactivity</th>
<th>Ratio C$^{14}$ in glucose to fructose moieties of sucrose</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Origin**</td>
<td>Sucrose</td>
</tr>
<tr>
<td>1</td>
<td>Sucrose-U-C$^{14}$</td>
<td>$6 \times 10^{-3}$</td>
<td>1540</td>
<td>2</td>
<td>98</td>
</tr>
<tr>
<td></td>
<td>Fructose-U-C$^{14}$</td>
<td>$7 \times 10^{-3}$</td>
<td>3780</td>
<td>4</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>Glucose-U-C$^{14}$</td>
<td>$7 \times 10^{-3}$</td>
<td>3180</td>
<td>17</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td>Glucose-U-C$^{14}$</td>
<td>$0.7 \times 10^{-3}$</td>
<td>3620</td>
<td>29</td>
<td>61</td>
</tr>
<tr>
<td>2</td>
<td>Fructosyl-U-C$^{14}$ sucrose</td>
<td>$7 \times 10^{-3}$</td>
<td>1810</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Fructose-U-C$^{14}$ glucose</td>
<td>$7 \times 10^{-3}$</td>
<td>4160</td>
<td>4</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>glucose + unlabeled</td>
<td>$7 \times 10^{-3}$</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* cpm at 5.5% efficiency, the efficiency of counting on chromatograms.

** C$^{14}$ remaining at the origin of chromatograms (see Methods and Materials section).
Distribution of Radioactivity in the Midrib as a Function of Time after Application of Fructose-U-C^{14}

0.03 ml of a $7 \times 10^{-3}$ M fructose-U-C^{14} solution ($21 \times 10^6$ dpm/0.1 ml) was supplied over a period of 10 minutes to the cut end of the midrib 60 cm from the dewlap. A $7 \times 10^{-3}$ M solution of unlabeled fructose was then supplied continuously until the leaf was harvested. The results shown are for the ethanol soluble compounds in the midrib section 10 to 15 cm below the point of application.

<table>
<thead>
<tr>
<th>Time after application commenced (min)</th>
<th>Total C^{14} in the midrib section (^*) (cpm)</th>
<th>Percent of total radioactivity</th>
<th>Ratio C^{14} in glucose to fructose moieties of sucrose</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Origin</td>
<td>Sucrose</td>
<td>Glucose</td>
</tr>
<tr>
<td>10</td>
<td>940</td>
<td>4</td>
<td>11</td>
</tr>
<tr>
<td>20</td>
<td>6900</td>
<td>2</td>
<td>10</td>
</tr>
<tr>
<td>60</td>
<td>9540</td>
<td>3</td>
<td>87</td>
</tr>
<tr>
<td>180</td>
<td>13200</td>
<td>2.5</td>
<td>96</td>
</tr>
</tbody>
</table>

\(^*\) cpm as in table I.

...continued at different times. At equal distances from the application zone the proportion of label in sucrose had increased from 11% after 10 minutes to 96% after 180 minutes (table II). During the period up to 20 minutes the ratio of the C^{14} in glucose plus fructose to the C^{14} in sucrose increased slightly with distance from the application zone (fig 2a). However, after 180 minutes the ratio had decreased to a low value at all distances from the application zone, approaching zero at distances of 20 cm and beyond (fig 2b). Most of the increase in the proportion of C^{14} in sucrose had occurred by 60 minutes.

We conclude that glucose and fructose can enter the translocation stream and are converted to sucrose during movement. Since virtually no label appeared in free glucose when fructose was supplied and vice versa, the hexoses cannot have been degraded to a common transit molecule which is in equilibrium with hexoses. The results presented so far are consistent with sucrose being a physiological transit molecule but they do not eliminate the possibility that the transit molecule is a breakdown product of sucrose such as a hexose or hexose derivatives.

Evidence That the Sucrose Molecule is Translocated. When fructosyl-U-C^{14} sucrose was supplied to an intact midrib essentially all the translocated radioactivity was recovered as sucrose, and there was no significant randomization of label between the hexose moieties (expt. 2, table I). Hence, either the sucrose molecule is translocated without cleavage, or there is no equilibration between the hexose carbons during a process involving the breakdown and resynthesis of sucrose. The latter possibility was eliminated by supplying an equimolar mixture of fructose-U-C^{14} and unlabeled glucose. With this sugar mixture the sucrose component of translocated radioactivity contained an equal amount of label in the glucose and fructose moieties (expt. 2, table III).

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**Table II**

**Distribution of Radioactivity in the Midrib as a Function of Time after Application of Fructose-U-C^{14}**

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**Fig. 2a and 2b.** Total radioactivity and the ratio of C^{14} in glucose plus fructose to sucrose in the midrib of leaves 20 minutes (2a) and 180 minutes (2b) after the application of fructose-U-C^{14}. Experimental details were as described in table III.

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Other experiments showed that when labeled glucose or fructose were supplied the sucrose component of translocated radioactivity was labeled equally in the hexose moieties irrespective of the concentration of supplied hexose (expt. 1, table I), the time after application (table I) or the distance from the point of application. This was true in spite of large differences in the amount of radioactivity in the free hexoses of the same midrib sections.

The simplest interpretation of these results is that in the conducting tissue sucrose and fructose are interconverted via the hexose phosphate pool during formation of sucrose. Clearly, the sucrose molecule once formed, must be translocated without further cleavage and resynthesis occurring.

**Translocation of Fructosyl-U-C\textsuperscript{14} Sucrose from the Leaf to the Sheath and Stem.** Our earlier studies (5) provided evidence that during the accumulation of sucrose into the storage compartment of immature storage tissue slices, sucrose is obligatorily hydrolyzed and resynthesized. Part of the evidence for this conclusion was that label appeared in the glucosyl moiety of sucrose accumulated from fructosyl-U-C\textsuperscript{14} sucrose. The following experiment was carried out to examine the translocation of sucrose to the sheath and stem, and to determine if our findings on the accumulation of sucrose by immature storage tissue discs were true for intact plants.

After supplying a fructosyl-U-C\textsuperscript{14} sucrose solution to the cut end of a midrib for 8 hours the radioactive compounds in the midrib, sheath, node, and internodal tissue below the node were examined (table III). By pressing the internodal tissue with a pestle a sample of juice was obtained consisting mainly of the contents of large, easily broken parenchyma cells. To obtain juice from conducting tissue further pressure was exerted on the tissue, the fibre residue washed with water, and the excess moisture removed by squeezing through muslin. The residual material consisted mainly of intact conducting tissue with adhering parenchyma cells and cell wall debris. The ratio of reducing sugars to sucrose for the parenchyma and conducting tissue extracts were 3.1 and 0.74 respectively indicating that a large degree of separation was achieved. Since virtually all the sugar in conducting tissue is sucrose the proportion of cross contamination of one fraction by another may be estimated from the levels of sucrose, glucose, and fructose in the total tissue and the parenchyma and conducting tissue extracts. It was calculated that approximately 25% of the sucrose in the internodal conducting tissue extract was derived from contaminating parenchyma cells.

During movement from the leaf to the stem there was little randomization of the label in the hexose moieties of sucrose (table III). The ratio of 0.03 for the C\textsuperscript{14} in the glucosyl to fructosyl moieties of sucrose in the internodal conducting tissue extract must represent a maximum value for sucrose actually in the conducting tissue since it is most distant from the point of application. This value becomes 0.01 when corrected for the contamination by parenchyma sucrose.

The ratio of 0.12 for C\textsuperscript{14} in the glucosyl to fructosyl moieties of parenchyma sucrose is a minimum value. It was not possible to estimate directly the amount of conducting tissue sucrose in the parenchyma extract. The corrected value for the ratio would be 0.16 and 0.19 if 5% or 10% respectively of the sucrose in the parenchyma extract was derived from conducting tissue. The randomization of label in the hexose moieties of parenchyma sucrose, and the relatively high proportion of radioactivity in glucose, fructose, and nonsugar compounds were con-

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**Table III**

<table>
<thead>
<tr>
<th>Tissue examined</th>
<th>Total radioactivity in tissue* (cpm)</th>
<th>Per cent of total radioactivity</th>
<th>Ratio of C\textsuperscript{14} in glucose to fructose moieties of sucrose</th>
<th>Specific activity of sucrose (cpm/ug)</th>
<th>Specific activity of hexoses (cpm/ug)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Midrib (5 cm above the dewlap)</td>
<td>17800</td>
<td>2</td>
<td>0.05</td>
<td>170</td>
<td>8</td>
</tr>
<tr>
<td>Sheath (5 cm above the node)</td>
<td>3460</td>
<td>3</td>
<td>0.03</td>
<td>5.6</td>
<td>1.2</td>
</tr>
<tr>
<td>Nodal tissue (1 cm length)</td>
<td>2090</td>
<td>9</td>
<td>0.06</td>
<td>3.4</td>
<td>0.12</td>
</tr>
<tr>
<td>Internodal tissue below node</td>
<td>1490</td>
<td>6</td>
<td>0.09</td>
<td>1.6</td>
<td>0.02</td>
</tr>
<tr>
<td>Juice from internodal tissue</td>
<td>670</td>
<td>14</td>
<td>0.12</td>
<td>0.8</td>
<td>0.03</td>
</tr>
<tr>
<td>Parenchyma</td>
<td>720</td>
<td>1</td>
<td>0.03</td>
<td>2.4</td>
<td>0.02</td>
</tr>
<tr>
<td>Conducting tissue</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*cpm as in table I.

** The procedure for the preparation of these fractions and the basis for regarding them as predominantly from parenchyma and conducting tissue is discussed in the text.
sistent with sucrose being hydrolyzed upon entry into the storage parenchyma.

We interpret these results to mean that the sucrose molecule is translocated intact through the leaf, sheath, and stem of the sugarcane plant. Our experiments do not exclude the possibility that the transit molecule is a sucrose derivative containing the sucrose moiety. In some plants sucrose is not the predominant component of phloem exudates (6,8) or translocated radioactivity following photosynthesis in C^{14}O_{2} (4,7). Nevertheless sucrose or a sucrose derivative could be the transit molecule in these cases.

**Summary**

By supplying sucrose labeled only in the fructose moiety to leaves, then examining the distribution of radioactivity in translocated compounds, conclusive evidence is obtained that the sucrose molecule remains intact during translocation through the vascular tissue of the leaf, sheath, and stem of the sugarcane plant. Sucrose is demonstrated as the predominant component of translocated photosynthetic. The translocation velocity for photosynthetic and supplied sugars was between 4 and 6 cm/minute. Randomization of label occurred during the movement of fructosyl-U-C^{14} sucrose from the vascular tissue into the parenchyma cells of the stalk indicating that sucrose is broken down and resynthesized during movement into storage.

**Addendum.** Recently Hartt, Kortschak, Forbes, and Burr (Plant Physiol. 38: 305-18, 1963) reported a very thorough study on the translocation of radioactive photosynthetic in sugarcane. As reported in the present paper most of the translocated radioactivity was found in sucrose in short-term experiments. We recorded somewhat higher translocation velocities both of photosynthetic and radioactive sugars supplied to the leaf midrib.

**Literature Cited**