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## Sugar Uptake and Translocation in the Castor Bean Seedling I. Characteristics of Transfer in Intact and Excised Seedlings<sup>1</sup>

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*Summary.* Changes in the dry weights of various parts of the castor bean seedling showed that the rates of transfer of material through the cotyledons to the embryonic axis exceeded 2 mg/hour after 5 to 6 days of germination. The sugar present in the endosperm was predominantly, and in the cotyledon almost exclusively, sucrose. Anatomical features were described which contribute to the efficiency of the cotyledons as organs of absorption and transmittal of sucrose to the embryonic axis, where hexoses are much more prevalent.

The ability of the cotyledons to absorb sucrose survived removal of the endosperm from the seedling. A series of experiments is described in which the cotyledons of such excised seedlings were immersed in sucrose-<sup>14</sup>C and measurements made of uptake and of translocation to various parts of the seedling. Increasing rates of absorption were observed as the sucrose concentration was raised to 0.5 M and these rates were maintained for several hours. Removal of the embryonic axis (hypocotyl plus roots) drastically altered both the response to sucrose concentration and the time course of absorption by the cotyledons.

More than 80% of the sugar normally entering the cotyledons from the endosperm is transmitted to the embryonic axis and this extensive turnover was seen also in pulse/chase experiments with excised seedlings. The cotyledons of excised seedlings absorbed sucrose against high apparent concentration gradients. The absorption was stimulated by phosphate and had a pH optimum at about pH 6.4. It was inhibited by arsenate, azide and 2,4-dinitrophenol.

Previous work in this laboratory (1) has elucidated the pathway by which fat in the endosperm of the castor bean seedling is converted to carbohydrate during germination, but the means by which this sugar is introduced into the cotyledons and transported to the growing embryonic axis is unresolved. The cotyledons are specialized absorptive organs which are wholly responsible for the uptake and transfer of the considerable substrate requirements of the heterotrophic seedling. This paper describes, first, features of the seedling's early growth and anatomy which seem to contribute to an understanding of the overall process of sugar uptake by the cotyledons. Information on the amounts and nature of sugar trans-

ported are then provided. Finally the characteristics of sucrose uptake by the cotyledons of excised seedlings (i.e. seedlings from which the endosperm had been removed) and features of long range transport are described.

### Materials and Methods

Castor beans (var. Cimmaron) were soaked overnight at room temperature and germinated in moist vermiculite in the dark at 30°. For the growth study, material was sampled from a large population of seedlings and dissected into endosperm (excluding the outer integuments), cotyledons, and embryonic axis (hypocotyl and rudimentary plumule plus roots). Fresh weights were determined and the tissue was dried for 3 days at 80° prior to measuring dry weight.

Anatomical study was made on seedlings germinated for 5 days, fixed in absolute ethanol/glacial

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acetic acid 3:1, embedded in paraffin and sectioned at  $5\mu$  on a microtome. For sugar analysis, the plant material was homogenized in hot 80% (v/v) ethanol. The filtered extract was dried under vacuum at  $40^\circ$ , treated with anhydrous ether to remove fats, redried and dissolved in a small volume of water. The aqueous extract was then passed successively through columns of Dowex 50- $\times$ 8 and 1- $\times$ 10 ion exchange resin, and made to a known volume. Aliquots were taken for reducing sugar determinations using Nelson's modification (7) of Somogyi's method. Sucrose was estimated as hexose following hydrolysis with invertase.

**Sucrose Uptake.** The apparatus supporting the seedlings consisted of a 5 ml cylindrical center well with 6  $10 \times 3$  cm polyethylene tubes arranged radially. The endosperm was removed from 5 days old seedlings and the plants arranged so that the exposed cotyledons of all of the seedlings (4-6) were immersed in the liquid in the center well, while the attached embryonic axes, including lateral roots, were placed individually in deionized water in the larger, outer tubes. The apparatus was provided with a lid and the whole assembly was kept under a light tight container.

Radioactive sucrose with a specific activity of 20 to 30 mc/mm, was suitably diluted and placed in the solution in the center well and uptake into the cotyledons was determined by assaying for  $^{14}\text{C}$  in samples removed at appropriate intervals from the solution. The solution was stirred thoroughly for at least 1 minute with a Pasteur pipette fitted with a rubber bulb before drawing off 5 replicate samples of 2  $\mu\text{l}$  or 10  $\mu\text{l}$  and transferring each into a vial containing 13 ml of scintillation fluid for counting. The scintillation fluid consisted of: 350 ml toluene, 350 ml dioxane, 210 ml methanol, 73 g naphthalene, 4.52 g PPO (2,5-diphenyloxazole) and 0.078 g POPOP [1,4-bis-2-(5-phenyloxazolyl)-benzene].

## Results

**Growth Data.** The data in figure 1 show the changes in weight of the different parts of the seedling during germination. At the outset the cotyledons have a greater dry weight than the embryonic axis but this situation is reversed after day 4. The loss in dry weight from the endosperm between days 4 and 7 is almost exactly matched by the increase in dry weight in the cotyledons hypocotyl and roots. Figures 1 and 2 indicate that while the cotyledons continue to gain in dry weight the embryonic axis grows much faster. Both systems show a maximum growth rate between 5 and 6 days after the start of germination at which time they are together gaining dry weight at over 2 mg per hour. In other experiments, rates of up to 3.2 mg per hour have been recorded, and allowing

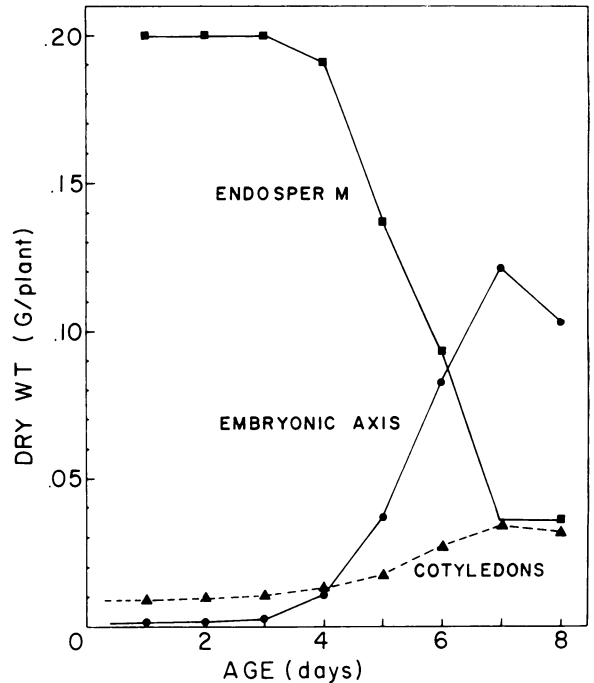


FIG. 1. Dry weight changes in the various parts of the castor bean seedling during germination at  $30^\circ$ .

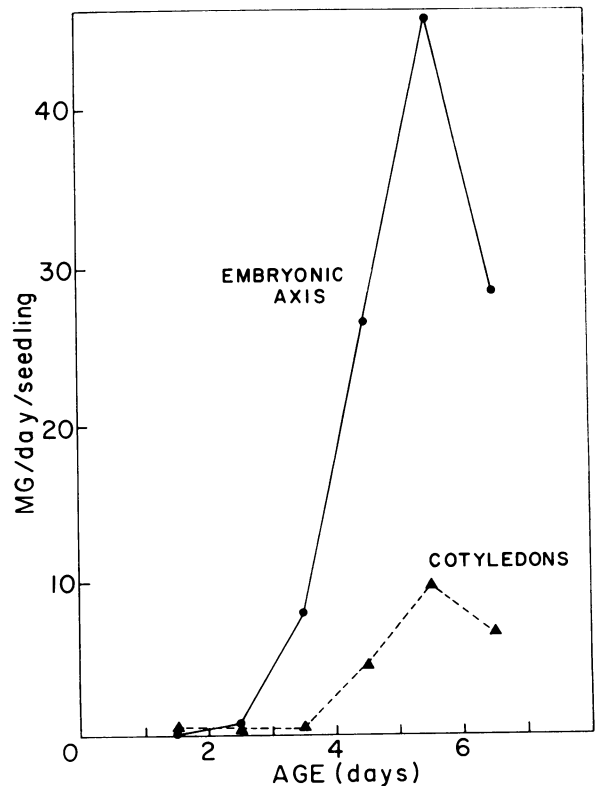
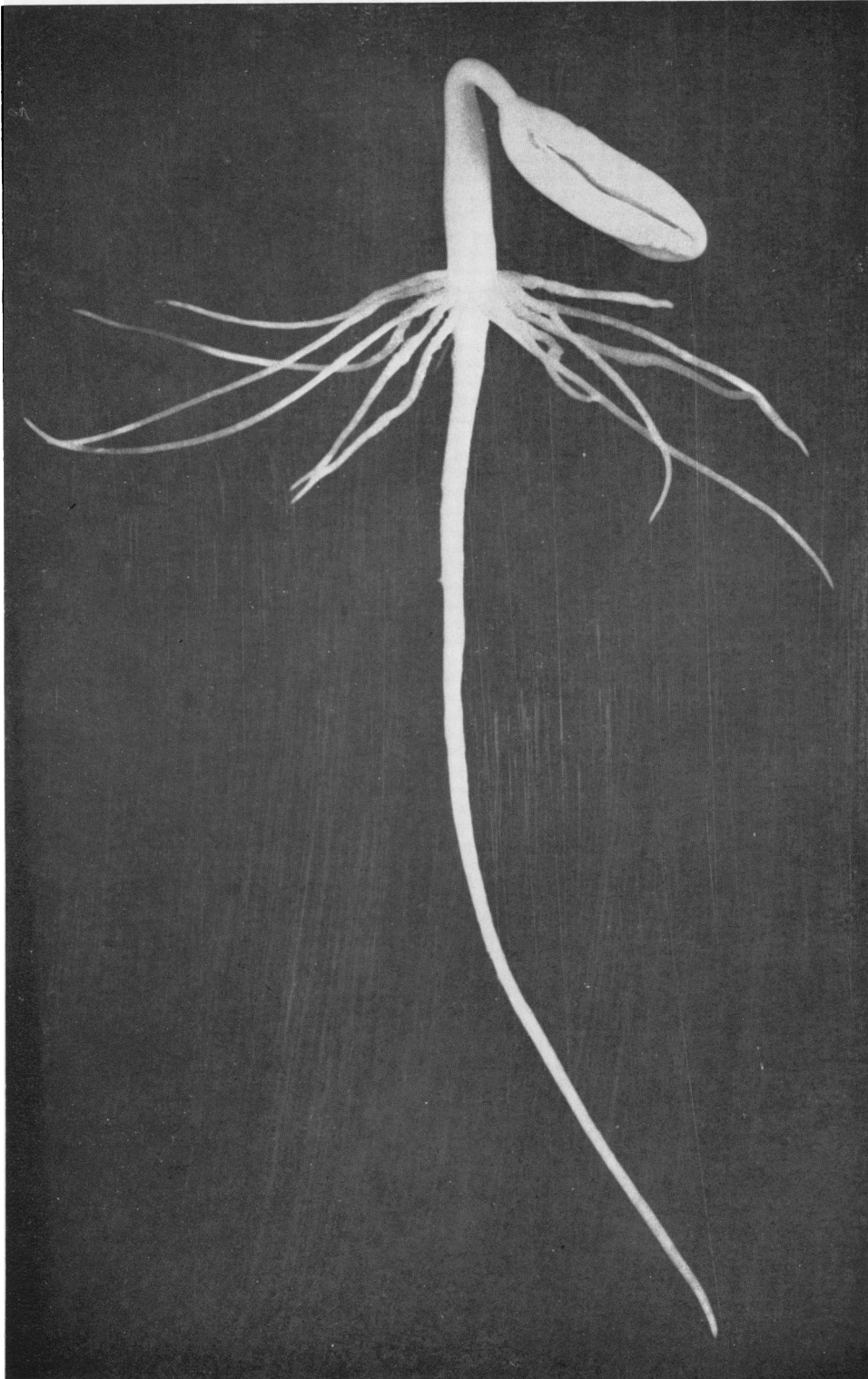


FIG. 2. Changes in rates of increase in dry weight of cotyledons and embryonic axis during germination.



I=1 Cm.

FIG. 3. The 5 days old castor bean seedling. At this stage the testa has been lost, the hypocotyl is still hooked and the cotyledons, with rudimentary epicotyl are enclosed within the endosperm. The endosperm is seen to be splitting along the plane of the enclosed cotyledons and it can be readily removed to provide the excised seedlings used in most of the experiments.

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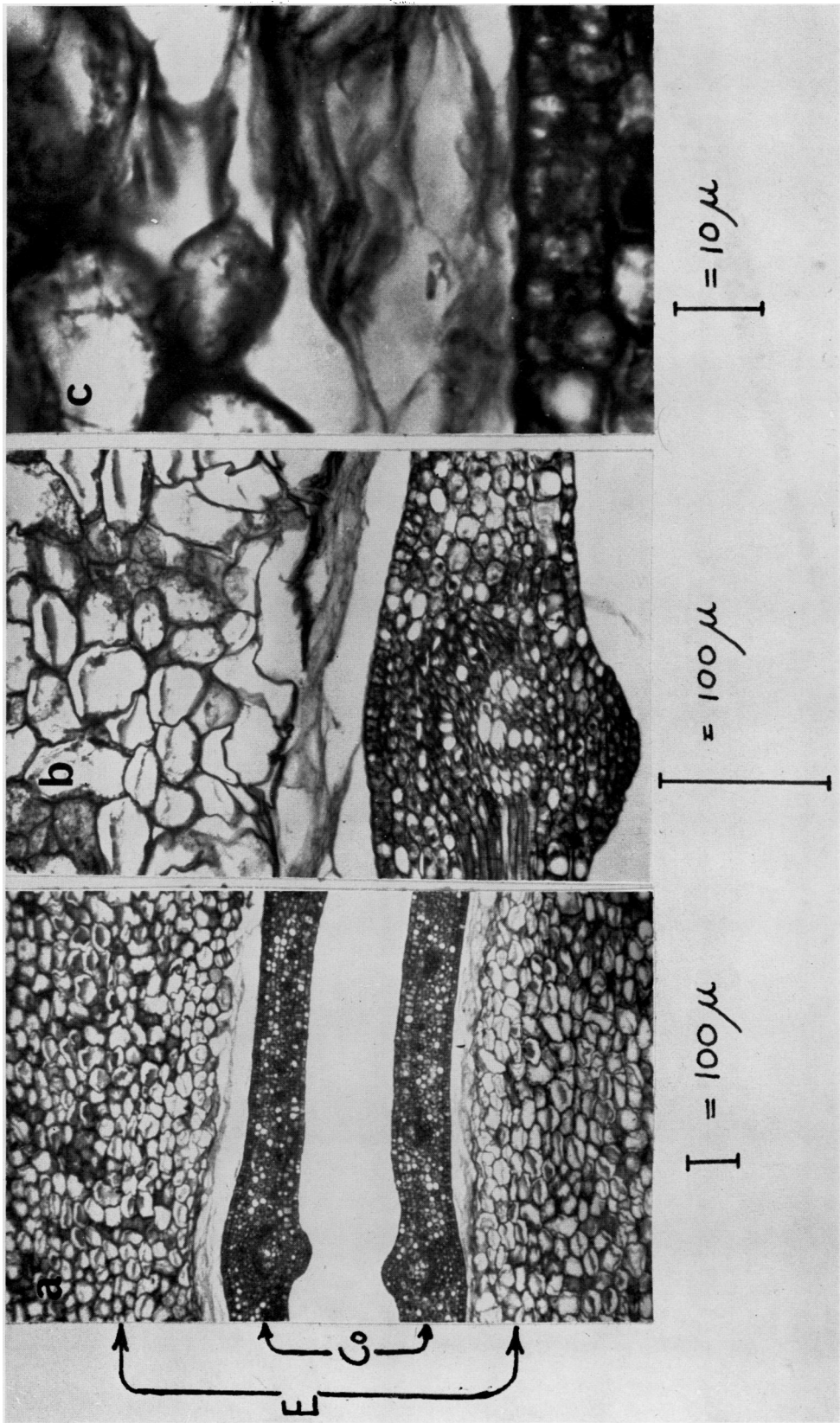


FIG. 4. Transverse sections through endosperm (E) and cotyledons (Co) as seen under the light microscope at different magnifications.

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for some respiratory losses, it seems clear that the endosperm must have the capacity to supply substrate, and the cotyledons the ability to absorb this material at rates in excess of 3 mg per hour.

**Anatomy.** The gross morphology of the castor bean seedling 5 days after the start of germination is shown in figure 3. The cotyledons occupy the mid plane of the bulky endosperm and are the only contact between endosperm and embryo. The internal structure of the cotyledons and the adjacent endosperm is shown in figure 4. The cotyledons are amply supplied with vascular bundles (see fig 4a) and material entering them from the adjacent endosperm has to traverse only 4 or 5 layers of mesophyll cells before encountering one of the many phloem elements that occur in a major vascular bundle (see fig 4b).

Substances passing from endosperm to cotyledons traverse a layer of non cellular material interposed between the 2 systems. Sections of fresh tissue show that the space between cotyledons and endosperm is completely occupied by material which is birefringent and which stains with Ruthenium Red. The intimate association between this layer and the tissue on either side is shown by the adhesions which remain on adjacent surfaces (endosperm and epidermal cells of the cotyledons) following shrinkage during fixation. One such zone is shown in higher magnification in figure 4c.

**Endogenous Sugar Levels.** Table I shows the results of sugar determinations in the various parts of 5 and one-half days old seedlings. At this stage large amounts of sugar, particularly sucrose, are present in the endosperm. Nevertheless the average sucrose concentration in the cotyledons is about twice that in the immediately adjacent endosperm, so that within the intact seedling at this stage, the cotyledons are able to absorb sugar efficiently despite an apparently unfavorable concentration gradient. The decrease in sucrose level from the outer to the inner portion of the endo-

sperm is presumably due to uptake by the cotyledons from the proximal region of the endosperm.

The amounts of reducing sugars in the endosperm are about one-fourth those of sucrose. In the cotyledons the preponderance of sucrose is even

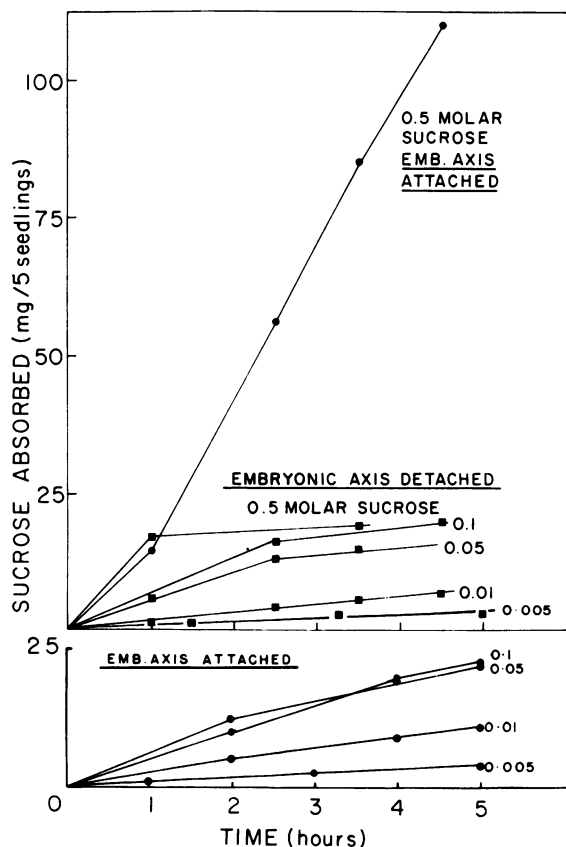


FIG. 5. Progress of sucrose absorption when cotyledons of excised seedlings were placed in solutions of different concentrations, and the effect of excising the embryonic axis from the cotyledons.

Table I. Amounts and Concentrations of Sucrose and Reducing Sugars in Various Parts of the 5 and One-half Days Old Castor Bean Seedling

	Sucrose		Reducing sugars	
	mg/g Fr wt	Molarity**	mg/g Fr wt	Molarity**
Outer endosperm	42.9	0.16	9.2	0.07
Inner endosperm (adjacent to cotyledons) (n = 2)*	24.7	0.09	6.4	0.04
Cotyledons (n = 12)	39.67	0.207	1.61	0.015
Hypocotyl (n = 6)	19.66	0.057	11.22	0.062
Roots (n = 6)	11.09	0.035	14.17	0.085

\* n = The number of separate measurements on which the mean data are based.

\*\* Based on the assumption that sugar present is distributed evenly throughout the measured amount of water in the tissue.

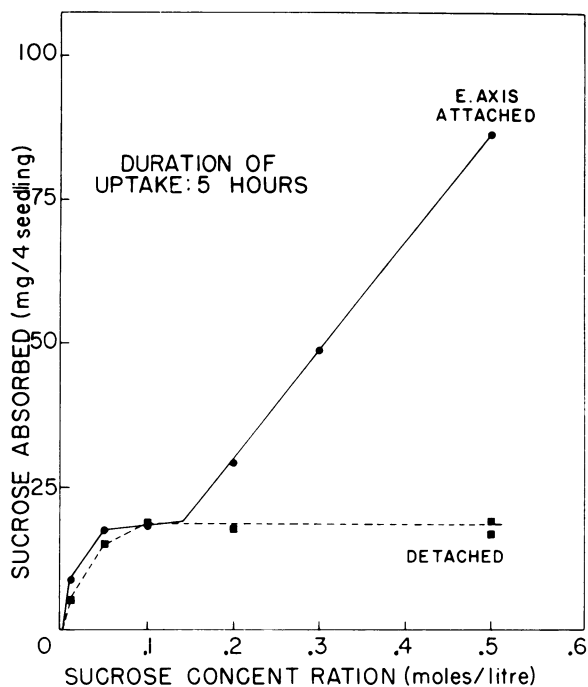


FIG. 6. Effect of concentration of sucrose on the absorption of sucrose by the cotyledons of excised seedlings and by cotyledons alone. (Uptake period, 5 hours)

greater; reducing sugars comprise less than 4% of the total. Moreover a sizeable fraction of this hexose is readily leached out when the endosperm is removed and the cotyledons are exposed to an aqueous medium. Thus 40% of the endogenous hexose appeared in the medium in 1 experiment while only 4% of the sucrose was leached out.

It is clear then that in the cotyledon, which is the initial site of uptake into the embryo and of loading into the translocation stream, almost all of the sugar is sucrose. By analyzing the exudate from seedlings whose embryonic axes had been excised at the point of hypocotyl attachment, an estimate was made of the concentration of sugars in the translocation stream leaving the cotyledons. Forty  $\mu$ l of exudate was collected from each of 10 seedlings and in the pooled material the concentration of sucrose was 0.07 M and that of reducing sugars 0.006 M.

In more distal parts of the embryonic axes of normal seedlings reducing sugars are much more abundant (table I). It thus appears that as in other systems, e.g. sugar cane (5), sucrose is the sugar actually transported and that hydrolysis occurs subsequently in tissues to which the sucrose is moved.

*Sucrose Uptake and Translocation by Excised Seedlings.* Figures 5 and 6, in which effects of concentration of exogenous sucrose and duration of exposure are shown, demonstrate that the excised seedlings are able to absorb sucrose rapidly through

their cotyledons; the remarkable capacity of these organs for sucrose uptake and transport in the intact seedling survives excision of the endosperm. Sucrose uptake was linear with time over a 100-fold concentration range up to 0.5 M (fig 5). When uptake by the excised seedling is plotted as a function of sucrose concentration (fig 6) it is clear, first, that the rate was progressively increased until quite high concentrations were reached (in different experiments these varied between 0.3 M and 0.5 M). Secondly, the curve is apparently biphasic, which would indicate saturation of a lower capacity absorption system at about 0.1 M sucrose. It is significant that the highest rates of absorption observed by the cotyledons after removal of the endosperm ( $> 4$  mg seedling/hr) exceed those which were deduced to occur in the intact seedling, when the attached endosperm is the source of

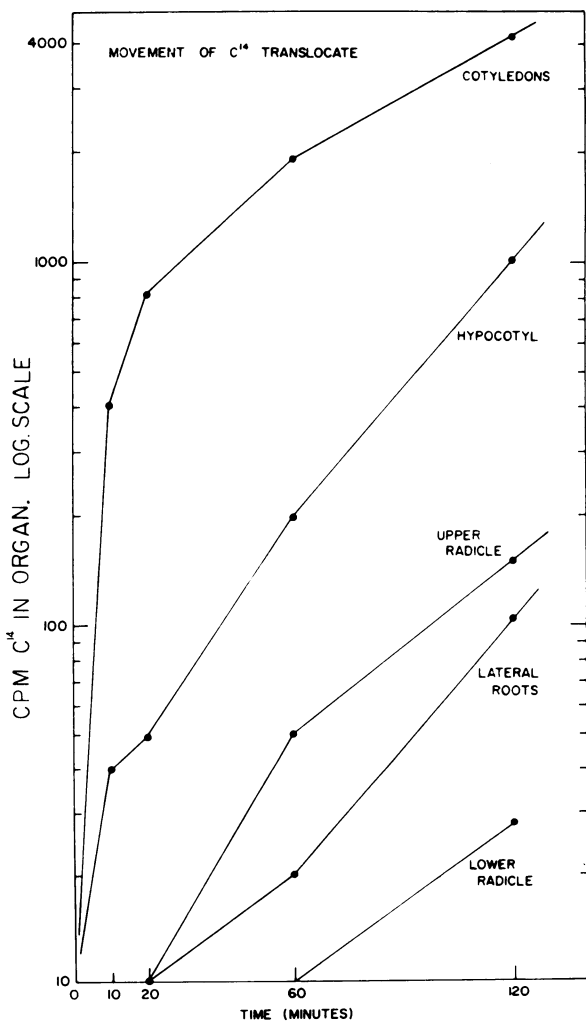


FIG. 7. Movement of  $^{14}\text{C}$  from sucrose- $^{14}\text{C}$  from cotyledons into various parts of the excised seedling. At time zero cotyledons were immersed in sucrose- $^{14}\text{C}$  (0.1 M) and seedlings sampled at the times indicated.

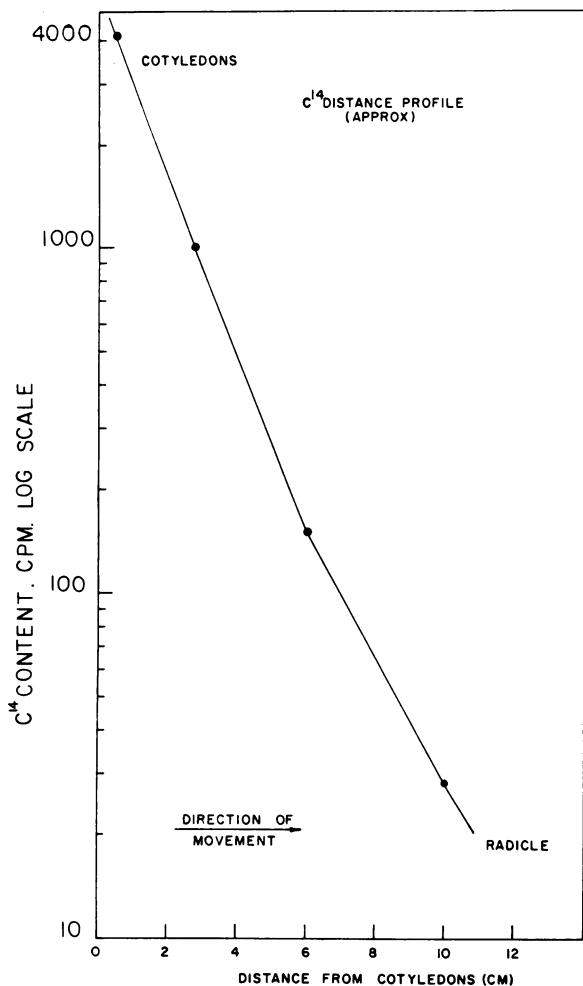


FIG. 8. The  $^{14}\text{C}$  distance profile in the radicle 2 hours after supplying sucrose- $^{14}\text{C}$  (0.1 M) to the cotyledons of excised seedlings.

translocating material. However these high rates were achieved from media with a concentration of sucrose considerably greater than that in the endosperm (approx 0.15 M). At this concentration the uptake by the excised seedling was about one-half that observed *in vivo*.

The influence of the embryonic axis on the uptake of sucrose by the cotyledons was examined by comparing the uptake by cotyledons alone to that observed when the cotyledons remained attached to the excised seedling (figs 5, 6). Uptake from 0.5 M sucrose by the cotyledons alone initially followed the pattern shown by the excised seedling. However the immediate sucrose requirements of the cotyledons were met within an hour and uptake was barely detectable after this time (fig 5). The uptake by the excised cotyledons in different levels of sucrose was similar to that of excised seedlings in concentrations up to 0.1 M, but further increases failed to stimulate uptake above this value.

It is clear then, that the embryonic axis strongly influences sucrose uptake by the cotyledons. Figure 7 shows that the axis provides a strong sink for sugar entering the cotyledons. The cotyledons of excised seedlings were immersed continuously in sucrose- $^{14}\text{C}$  and at intervals over 2 hours seedlings were removed and the  $^{14}\text{C}$  present in 80% ethanol extracts of various parts was measured. The rapid incorporation of  $^{14}\text{C}$  into the cotyledons was closely followed by a logarithmic rise in  $^{14}\text{C}$  in the hypocotyl. After 20 minutes  $^{14}\text{C}$  appeared in the upper radicle and lateral roots, and the lower radicle, which was 10 cm from the cotyledons, became radioactive after 1 hour. On completion of this 2 hour experiment the  $^{14}\text{C}$  distance profile was logarithmic (fig 8).

The turnover of sucrose in the cotyledons is demonstrated by the pulse/chase experiment shown in figure 9. By the end of the experiment, 8 and one-half hours after the pulse of sucrose- $^{14}\text{C}$ , the  $^{14}\text{C}$  in the cotyledons had fallen from its initial value of 93% of the total  $^{14}\text{C}$  in the seedling to a value of 26%. At this time the hypocotyl and

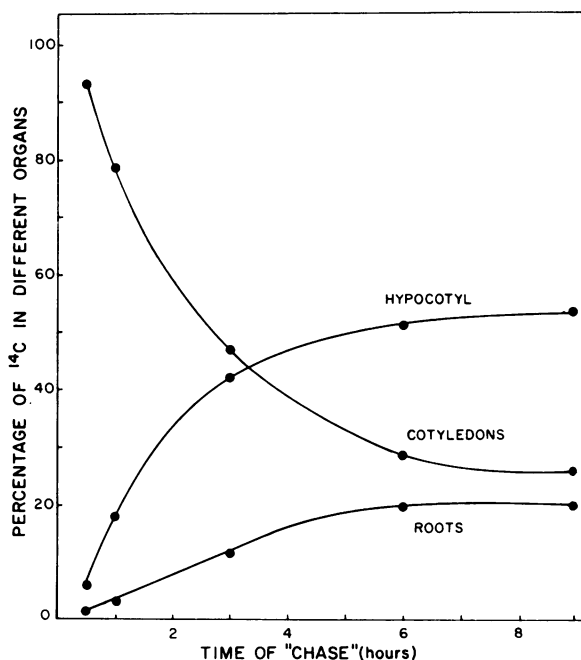


FIG. 9. Changes in the relative soluble  $^{14}\text{C}$  content of different parts of the excised seedling after immersing the cotyledons for 30 minutes in sucrose- $^{14}\text{C}$ . The cotyledons of 5 excised seedlings were immersed in 0.1 M sucrose- $^{14}\text{C}$  for 30 minutes (the pulse). The medium surrounding the cotyledons was then replaced with unlabeled sucrose. At this time 1 seedling was removed, rinsed for 5 minutes in running water, separated into its component parts and the total soluble  $^{14}\text{C}$  in each part was determined. The 4 remaining seedlings were removed at the designated times during the chase and treated similarly. The  $^{14}\text{C}$  in each organ is expressed as percent of the total soluble  $^{14}\text{C}$  in the seedling.

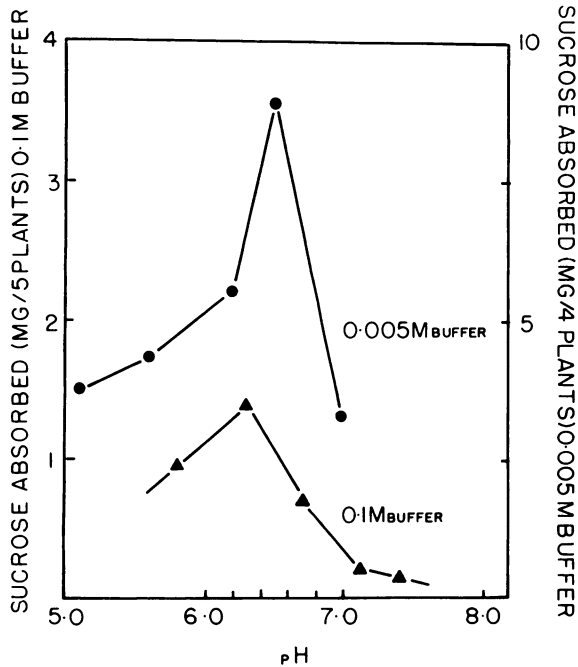


FIG. 10. Effects of pH and buffer concentration on sucrose absorption by the cotyledons of excised seedlings. (Uptake period, 5 hrs)

root system accounted for 54% and 20% of the total.

The above experiments demonstrate that the major fate of sucrose absorbed by the cotyledons is transfer to the translocation system, while a much smaller fraction is retained in the cotyledons.

*The Active Nature of Sucrose Absorption.* Various features of the absorption system point to a dependence on metabolic energy.

A) Accumulation against a concentration gradient: Excised seedlings absorbed sucrose at a constant rate, which was sustained for at least 5 hours from solutions as dilute as 5 mM (fig 5). At the outset of such an experiment the sucrose concentration in the aqueous phase of the cotyledons is roughly 0.2 M. It thus appears certain

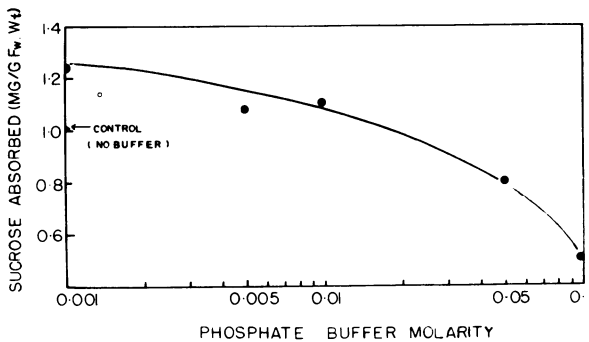


FIG. 11. Effect of phosphate concentration (pH 6.2) on sucrose absorption. Seedlings exposed to 0.1 M sucrose for 5 hours.

that the absorption does in fact occur against a concentration gradient.

B) Effect of pH: The data of figure 10 demonstrate that sucrose absorption by excised seedlings is strongly dependent upon the pH of the medium surrounding the cotyledons at a sucrose concentration (5 mM) that would necessitate some form of active mechanism for sustained rates of sugar uptake. Conceivably differences in pH could induce physicochemical changes in the tissue which might influence its permeability to sucrose, but the sharp dependence on pH speaks against a purely passive entry.

C) The effect of phosphate concentration: The data in figure 10 show a fairly narrow optimum pH range (6.3-6.5) for sucrose uptake, despite large differences in phosphate buffer concentration, but they also reveal a strong inhibition of uptake at the higher concentration of buffer. More detailed information on sucrose absorption from 0.005 M solution at pH 6.2 and over a wide range of buffer concentration is shown in figure 11. There was a clear enhancement by 1 mM phosphate over the control rate in water, while increasing phosphate concentration progressively lowered sucrose absorption until at 100 mM the rate was reduced by 64%.

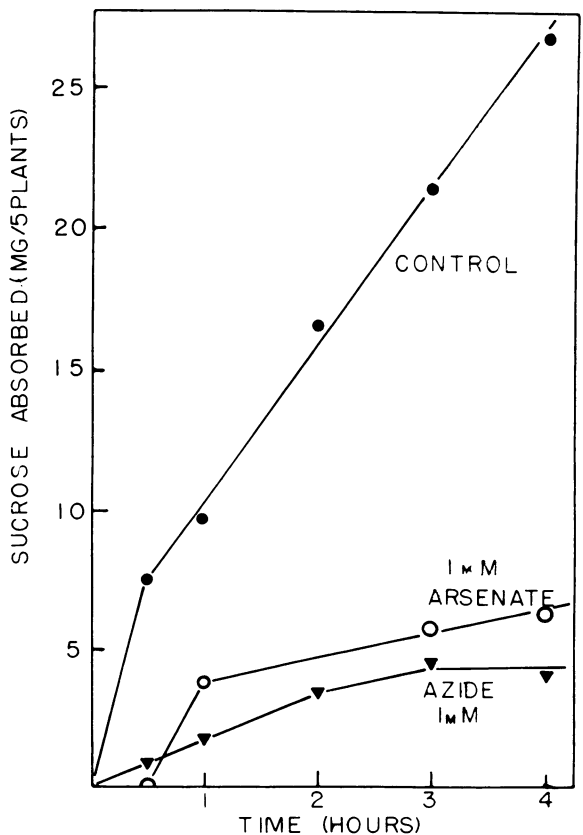


FIG. 12. The effect of azide and arsenate on sucrose absorption. Sucrose concentration, 0.1 M.



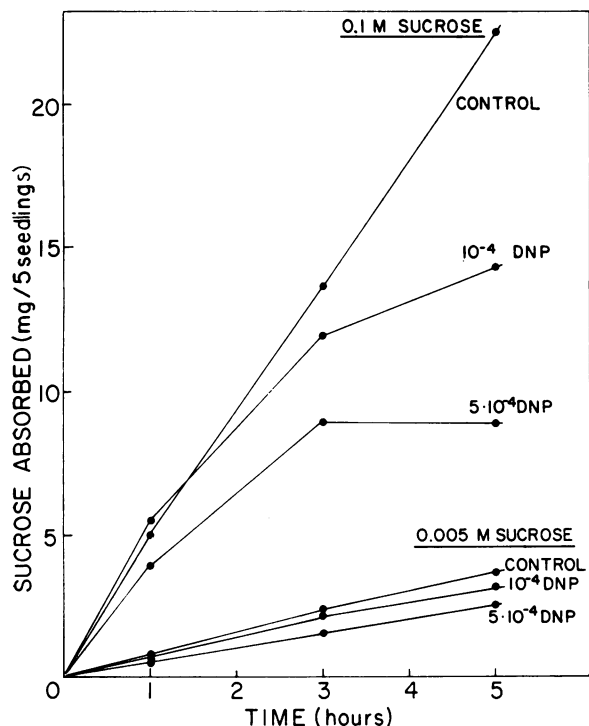


FIG. 13. The effect of DNP on the progress of sucrose absorption from solutions of different concentrations.

D) Metabolic inhibitors: Sodium arsenate and sodium azide at 1 mM concentration inhibited sugar uptake from 0.1 M sucrose by 77% and 85% respectively over a 4 hour period (fig 12).

Uptake from 0.1 M sucrose was also inhibited by 2,4-dinitrophenol (DNP) at both 0.0005 M and 0.0001 M (fig 13). Decreasing the concentration to 0.0001 M restored uptake to the control rate. At 0.005 M sucrose, DNP also inhibits the uptake, but the effect is far less striking than that observed with the higher sucrose concentration.

The turnover of sucrose absorbed by the cotyledons from the medium is also inhibited by DNP (fig 14). The cotyledons of excised seedlings were initially exposed to unlabeled sucrose for 1 hour and then pulsed for 30 minutes with sucrose- $^{14}\text{C}$  in either deionized water or in 1 mM DNP, followed again by unlabeled sucrose (in 1 mM DNP for the second treatment). Sucrose concentration was 0.1 M throughout these operations. The cotyledons of control seedlings showed a very rapid incorporation of  $^{14}\text{C}$  during the pulse, which was lost rapidly during the subsequent chase. The fall was accompanied by a steady increase in the  $^{14}\text{C}$  present in the hypocotyl and subsequently in the root system. The presence of DNP in the medium around the cotyledons during the initial pulse and the following chase had 2 pronounced effects on the uptake and translocation profile.

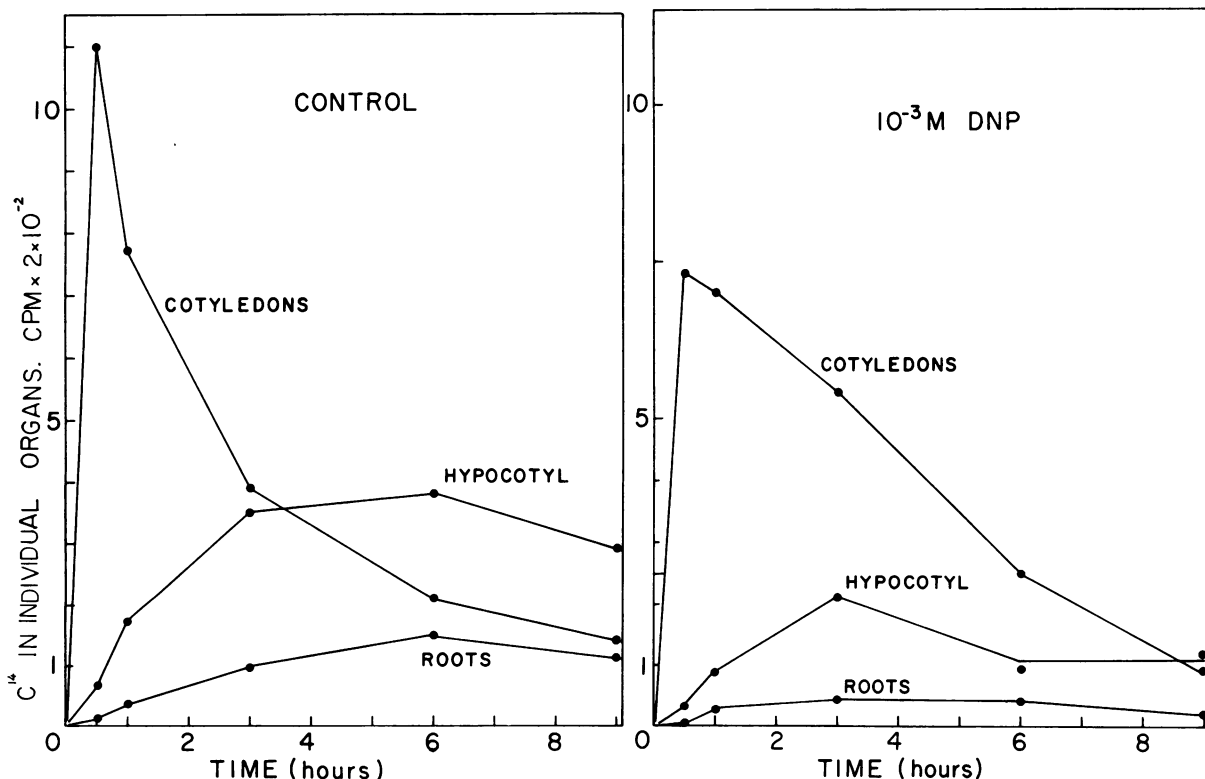


FIG. 14. The effect of DNP (0.001 M) on the absorption and redistribution of  $^{14}\text{C}$  from sucrose- $^{14}\text{C}$  supplied during a 30 minute pulse. DNP was present with sucrose- $^{14}\text{C}$  during the pulse in the treated seedlings. At 30 minutes the controls were transferred to 0.1 M unlabeled sucrose and the seedlings previously exposed to DNP were transferred to 0.1 M unlabeled sucrose containing DNP.

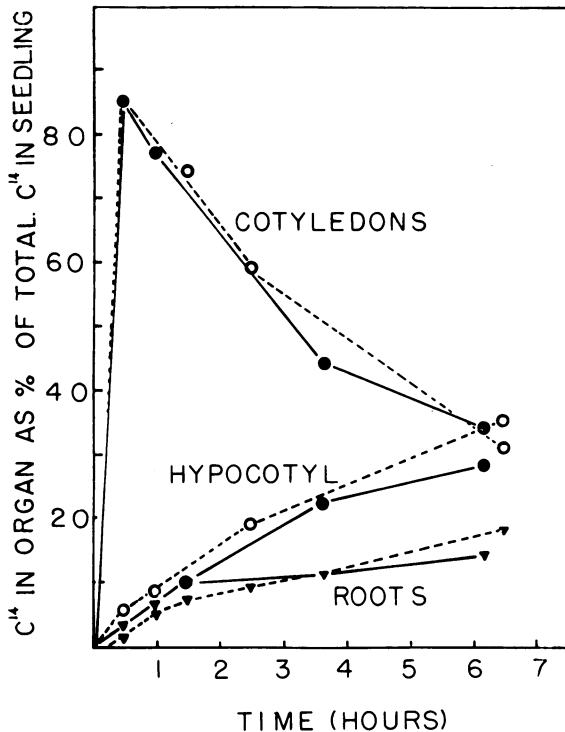


FIG. 15. The effect of DNP, added after the pulse, the redistribution of  $^{14}\text{C}$  from sucrose- $^{14}\text{C}$  absorbed during a 30 minute pulse. No sucrose was provided after the pulse. The dashed curves refer to the DNP treated samples.

Firstly the DNP reduced the  $^{14}\text{C}$  incorporated into the cotyledons during the initial pulse by 40%. Secondly, movement of  $^{14}\text{C}$  out of the cotyledons over the first 2 hours following the pulse was reduced by about 50%.

It is suggested that the reduction in movement out of the cotyledons is a consequence of reduced incorporation into them rather than a direct effect of DNP on the translocation system for the following reasons. Firstly, by the end of the experiment the  $^{14}\text{C}$  in the cotyledons had fallen to about 1000 cpm irrespective of treatment. If DNP had been exerting a direct effect on movement out of the cotyledons a greater retention of label would have been expected. As it was, 87% was removed in the control, and 81% in the DNP treatment. Secondly, the time taken for the  $^{14}\text{C}$  to reach the hypocotyl and subsequently the root system, was unaffected by DNP; hence we presume there was no delay in moving the  $^{14}\text{C}$  along the vascular system of the cotyledon. Thirdly, DNP caused an appreciable increase in the amount of  $^{14}\text{C}$  that leaked out of the cotyledons back into the medium following the pulse. This effect lends support to the notion that uptake into the vascular system is the site of DNP action.

This suggestion gains force from results shown in figure 15. Seedlings were pulsed for 30 minutes

with sucrose- $^{14}\text{C}$  as previously, but DNP was not added until the start of the chase, and furthermore, in this experiment no sucrose was added during the chase; the cotyledons remained in either water or in 1 mM DNP. If the inhibitor was exerting a direct effect on transport of sucrose in the vascular system, some lowering of the rate of loss of radioactivity from the cotyledons to the remainder of the seedling would be expected. As is clear from figure 15, DNP had no effect on any feature of the distribution of radioactivity in the seedling following the pulse. Extrapolation of the present data reveal that by 8 hours about 60% of the activity present in the cotyledons immediately after the pulse would have been lost, compared with over 80% in the previous experiment where unlabeled sucrose was used for the chase.

### Discussion

The rapid phase of increasing dry weight of the embryonic axis of the germinating castor bean seedling, beginning on day 4, occurs at the ultimate expense of fat present in the endosperm. The first stage in this process is the conversion of fat into sugars (with sucrose as the primary product) and the second the absorption and transmittal of these sugars to the embryonic axis by the cotyledons. From the dry weight changes described (figs 1, 2) it is clear that between days 5 and 6 the cotyledons transmit more than 2 mg of material/hour. Although the analytical data (table I) show that hexoses are present in the endosperm [and these are absorbed by the cotyledons (8)] they are present in only minute amounts in the cotyledons and it seems clear that sucrose is the material moved in the translocation stream.

The anatomical features of the cotyledons (fig 4), particularly their thinness and the density of distribution of their vascular bundles, apparently fit them for their role in collecting sucrose and loading it into the transport mechanism. The cotyledons do not function exclusively in this role, since they also gain in dry weight while the embryonic axis is growing. However the extent of this local demand for substrate in the cotyledons themselves is, at best, only a small fraction of the total entering them. Thus, in the experiment described in figure 2, when the amount of sugar entering the cotyledons was over 2.4 mg/hour the flux between endosperm and embryonic axis was 2 mg/hour and only 0.4 mg/hour were consumed in the growth of the cotyledons.

This feature of rapid transport of the bulk of the entering sugar makes the system an unusually good experimental material with which to study the translocation process, particularly since, as we have shown, the seedling with its attached cotyledons can be easily removed from the endosperm and it retains its high capacity for sugar absorption

and transmittal to the embryonic axis. Translocation is frequently measured by exposing leaves to a pulse of  $^{14}\text{CO}_2$  in the light and measuring the subsequent appearance of  $^{14}\text{C}$  in other regions. Frequently in such experiments a relatively small fraction of the  $^{14}\text{C}$  moves out of the treated leaf (3, 6, 9, 10). A valuable feature of the castor bean system is that this fraction is large; as shown in figure 15, 80 % of the  $^{14}\text{C}$  absorbed from sucrose- $^{14}\text{C}$  during a 30 minute pulse moved out of the cotyledons during the subsequent 8 and one-half hours. The velocity of translocation, roughly 5 cm/hour is far below the highest rates reported (100-300 cm/hr) for example by Hartt et al. (4) in sugar cane leaves and by Webb and Gorham (10) in squash petiole, but is somewhat higher than that observed in *Salix* stem (0.2-2.0 cm/hr) by Canny (2).

Progressively larger amounts of sucrose were absorbed at steady rates over several hours as the sucrose concentration bathing the cotyledons was increased to as high a value as 0.5 M. There is evidence for saturation of a low capacity absorption system at about 0.1 M, and from the fact that removal of the embryonic axis abolished the capacity of the cotyledons to respond to higher concentrations of sucrose we conclude that the lower capacity system represents utilization within the cotyledons themselves as distinct from transfer into the translocation system. Conceivably the bulk of the sugar absorbed from sucrose solutions of concentrations higher than 0.1 M passes rather directly to the phloem elements which have a high absorption capacity so long as the sink represented by the embryonic axis is present. What is clear is that although the uptake of sucrose by attached cotyledons responds to increasing concentration over a wide range in a linear manner, the uptake is nevertheless under metabolic control. There is a clear

pH optimum and a response to phosphate concentration, and the uptake is inhibited strongly by azide and arsenate. 2,4-Dinitrophenol also inhibits absorption, and it was shown that this was not due to an effect on the translocation system itself.

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